The Quantification of Occupational Pesticide Exposure and Associated Effects on Human Health, with Special Consideration for Social Determinants

by

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Dedication

This dissertation is dedicated to my mother, Ms. Carolyn D.H. Beckett. Thank you for your love, support, and wisdom throughout the years.

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Abstract

Researchers first linked pesticide exposure to cancer development more than 50 years ago. Overall, exposure to pesticides such as chlorpyrifos (CPF) and other organophosphate pesticides (OPP) have been associated with increased risk of numerous cancers such as colorectal, esophageal, brain, and lung cancer. Today it is estimated that in low and middle income countries (LMICs), 2 to 8% of all cancers are thought to be due to occupational carcinogen exposure[6]. Pesticides, especially those persistent in the environment, such as organochlorine pesticides, have also been associated with endocrine disruption that leads to perturbations in metabolism, puberty, and birth defects. Farmworkers and especially migrant workers face unique barriers to healthcare, personal protective equipment, and often have increased exposure, however, the literature is lacking when it comes to quantifying pesticide exposure and understanding the social determinants associated with these exposures. Moreover, there needs to be more attention to specifically how these differences in exposure by social determinants can lead to changes in the human body.

This dissertation takes a multi-disciplinary approach to ascertaining pesticide exposure by occupation and understanding the resultant health effects in Thailand, and by quantifying pesticide biomarker and biological activity by farmwork history and citizenship status in the US . The second chapter of this dissertation presents a cross-sectional study completed in Chiang Rai, Thailand with conventional farmworkers and non-farmworkers. In this chapter, pesticide exposure was quantified through air samples and liquid chromatography/mass spectrometry.

Differences in self-reported health outcomes, complete blood counts and cholinesterase activity were assessed before and after pesticide spraying. In Chapter 3, the National Health and Nutrition Examination Study (NHANES) publicly accessible data was queried from 1999-2014 for pesticide exposure biomarker concentrations among farmworkers and non-farmworkers by citizenship status. Next in Chapter 4, NHANES data from Chapter 3 was combined with publicly available toxicity assay data from the US Environmental Protection Agency's (EPA's) Toxicity Forecast Dashboard (Toxcast). By linking human population exposure data in NHANES to dose-response data from Toxcast, we estimated adverse biological effects that occur across a range of human population-relevant pesticide doses.

In the Chapter 2 study, we detected the pesticides methomyl, ethyl chlorpyrifos, and metalaxyl via personal air sampling. Farmworkers in Northern Thailand had significant alterations in stress measures and clinical biomarkers, including decreased blood cell counts and cholinesterase activity, relative to matched controls. These changes were associated with occupational pesticide exposures. None of the farmworkers wore standardized PPE for the concentrated chemicals they were sprayed. Improving PPE use in LMICs presents a likely route for preventive intervention. Based on Chapter 3 NHANES outcomes, disparities exist in pesticide exposure by farmwork history and US citizenship present. Citizenship status and farmwork history were significantly associated with increased exposure for specific pesticides. In Chapter 4, NHANES participants are exposed to biologically active pesticide exposure concentrations based on the Toxcast *in vitro* assay testing data. In these last two aims, we found that nearly all participants had pesticide biomarkers in their urine or blood, and non-citizen farmworkers are more likely to be exposed to all of the pesticides except for 2,5-Dichlorophenol, 2,4-Dichlorophenol, and DEET acid. The results from Chapters 2, 3, and 4 highlight the need for additional research and legislation to reduce these gaps in pesticide exposure and health outcomes.

Chapter 1 Occupational Pesticide Exposure and Health Outcomes

1.1 Introduction

"Pesticide" is a broad term for the chemicals used to exterminate unwanted organisms harmful to cultivated plants or animals. Although rodenticides, herbicides, fungicides, and insecticides can harm human health, insecticides are the pesticide category most associated with negative human health effects [11]. Pesticides were first used in 1910 and by the 1940s were commonly used globally to tend to crops, home lawns, or rid people of pests at the beach [12]. The pesticide revolution seemed like the first step towards the guarantee of a future that depended on science to improve the lives of people across the globe. However, the pesticide revolution has not been without consequences. Pesticide exposure has caused poisonings, oxidative stress, birth defects, reproductive disorders, and cancer in humans, but has also contaminated the environment and harmed animals in their natural habitats [13].

1.1.1 US Law and Pesticide Regulations.

The United States would see its first regulations for pesticides in 1947 with the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). FIFRA was the first act in the U.S. created to regulate pesticide branding to protect applicators, consumers and the environment [12]. It would not be until the 1962 publication of Silent Spring by Rachel Carson that you would begin to see attention turn to the subject of human health and the environment surrounding pesticides [14]. Silent Spring was a jolt to U.S. politics and climate change activism. In the late 1970s and throughout the 1980s, many things were happening legally for farmworkers in the United States. While there were wins and losses at the federal level for pesticide law, some states like California, were more incentivized to improve the health of people working with these chemicals. Cesar Chavez, an Arizona resident and descendant of Mexican immigrants, was credited with co-leading a massive movement to unionize farmwork in the United States, especially for the sake of the many migrant workers who were being sent to the United States for work with little support, pay, and dangerous working conditions [15]. Dolores Huerta and Cesar Chavez's efforts brought about the United Farm Workers Association (UFW) in the 1970s [15]. Through this collective, UFW was able to push the California government to create laws focused on improving Latino and Hispanic immigrants' health and the well-being of all farmworkers [15].

In his now famous Wrath of Grapes Speech in 1968, Mr. Chavez asserts that he and his fellow farmworkers first recognized the lingering effects of pesticides among themselves and their children [15]. California farmworkers were essential for getting persistent chemicals like DDT, DDE, and Dieldrin banned from use in California prior to the federal government acting on the known human harms of pesticides [15]. Unfortunately, efforts to restrict organochlorines also coincided with an increase in organophosphate poisonings among California growers [15]. Using compelling statistics and data on pesticides contributing to increased cancer, birth defects, and mortality rates, UFW was able to mobilize farmworkers, college students, and law makers [15]. After the successful boycott of the grape growing industry in 1970, California would go on to pass the 1975 California Agricultural Labor Relations Act [15].

The U.S. federal government passed the 1976 Toxic Substances Control Act (TSCA) to assist the U.S. government in better understanding how chemicals affect the human body [16]. Unfortunately, this legislation did not give the U.S. Environmental Protection Agency (EPA) the ability to require companies to complete research prior to registering (e.g. something similar to the FDA approval pipeline) [16]. In 1994, the North American Free Trade Agreement (NAFTA) would be the start of a decades long surge of seasonal farmworkers, often migrant workers from Mexico, to the United States and Canada [17].

Historically proprietary pesticides have been released to the global market with limited human safety testing, to later be banned due to post-market human health data revealing toxicity [18]. To increase the throughput of toxicological risk assessment for chemicals like pesticides, in 2008, the US EPA launched the National Toxicology Program and collaborated with multiple other federal agencies including the Food and Drug Administration and the National Institute of Environmental Health Sciences to create the Toxicology in the 21st Century (Tox21) program [19]. Through Tox21, researchers have been tasked with developing rapid testing methods, to determine the safety of chemicals such as food additives and pesticides. The aims of Tox21 are to understand the biological mechanisms that chemicals alter, to create a prioritization for chemicals to be tested and to create a wealth of data that can more accurately predict *in vivo* toxicological responses in the human body [19]. There are currently 85,000 chemicals on the global market that TSCA has listed in its inventory of substances, and there was little to no experimental toxicology or epidemiology data on most of them [20].

1.1.2 Biologically Monitoring Pesticide Exposure

To inform public health policy, research studies have been completed to understand the absorption, distribution, metabolism, and excretion (ADME) and exposure concentrations across a population. ADME describes the process that a toxicant undergoes in the human body from exposure to elimination of the toxicant from the human body [21]. Absorption refers to the method that the toxicant enters the human body, which can be through ingestion, inhalation, or dermally [21]. Distribution refers to how the toxicants move through the human body, and metabolism refers to how the human body breaks down the toxicants that are in the human body [21]. A major component of metabolism to understand, especially for chemical toxicants, is the concept of biotransformation which refers to the alteration of chemicals by different proteins within the human body to add or remove chemical groups of toxicants [21]. The purpose of biotransformation is to make the chemicals more easily excreted from the human body, however in transforming toxicants into their metabolites, many toxicants can become more biologically active within the human body [21]. Finally, excretion or elimination refers to how the chemicals are removed from the human body often through urine, feces, and also out of circulation through storage in the liver or adipose tissue [21,22]. To estimate the exposure of an individual to pesticides, it is common to measure pesticide parent compounds or metabolites in blood or in urine [21,22]. These indicative measures are often referred to as exposure biomarkers [21,22].

Although ADME studies are mandatory in agrochemical registration and development, the process for assessing this can be long and expensive [23]. ADME studies in agrochemical research are often focused on non-target toxicity to organisms like mammals, fish, non-pest invertebrates especially though soil and water contamination not effects on human health [23].

Most importantly, pesticide potency has been traditionally determined by intrinsic activity, the ability to bind to the target site, and bioavailability which is the ability of the chemical toxicant to reach the target site [24]. Many ADME studies use traditional toxicological study methods such as animal research (*in vivo*) using insects or *in vitro* using insect enzymes [24].

Researchers in academia, government, and industry are also using *in silico* computer modeling, recombinant enzymes, HPLC-high-resolution mass spectrometry, and agrokinetics in pesticide development [24]. *In silico* research was first seen at an industry-level in the 1980s in the pharmaceutical industry, roughly 30 years after these methodologies were developed and used to understand ADME toxicology [25,26]. Many researchers are looking at the possibility of using artificial intelligence, specifically machine learning, to better and more efficiently understand ADME toxicology of agrochemicals [25]. Using machine learning presents great opportunities for efficiently understanding ADME, but also great challenges especially due to the limited information on pesticide exposure and bioactivity in the literature [25]. Using these excretions from people, toxicologists can estimate pesticide exposure or subsequent health harms through measuring metabolites and other molecules called biomarkers.

1.1.3 Mechanisms of Action.

Pesticides work through a broad range of mechanisms, often relevant to their mechanism of action for targeting pests. Dichlorodiphenyltrichloroethane (DDT) was a once commonly used organochlorine which is environmentally persistent, and in many regions today with high vector-borne disease burdens DDT is still used to eradicate insects such as mosquitoes [27,28]. Organochlorines, such as DDT, alter the normal function of sodium channels by blocking normal γ-aminobutyric acid (GABA) protein function by attaching to the A complex of the ionosphere protein complex which results in an overaccumulation of chloride in the synaptic gap [9,29]. Barbiturates and benzodiazepines also inhibit GABA to induce sedation or anti-anxiety effects on humans [29]. Other chlorinated insecticides have also been found to compete for the picrotoxinin site at which t-butylbicylophophorothionate [29]. While organochlorines include a diversity of chemical structures, there are some similar symptoms of organochlorine poisoning in people such as impaired neural function within 2 to 8 hours of exposure that often first looks like a lack of activity until it results in tremors and convulsion [29].

Chlorpyrifos (CPF), an organophosphate, is absorbed dermally or inhaled for workers and more commonly by ingestion in the general population[30]. CPF is very lipophilic and therefore stored in fat, although the bioactive form of CPF, Chlorpyrifos-oxon (CPO), is not as lipophilic [30]. CPO acts as an insecticide by irreversibly binding to the enzyme cholinesterase [30]. CPF and CPO metabolites are excreted from the body through the urine and breast milk[30]. CPF is absorbed dermally or inhaled for workers and more commonly by ingestion in the general population[30]. In Meuling et al. 2005, two separate groups of three healthy males were exposed to either 5mg or 15mg of CPF dermally [31]. When exposing the skin of the subjects was directly wiped with water and cotton, 42-67% if the CPF was removed suggesting that washing any exposed skin directly after work can greatly reduce exposure levels [31]. Additionally, organophosphates, like CPF, are biotransformed within the body by phase I and II xenobiotic metabolizing enzymes like CYP450s into oxygenated and more bioactive chemicals such as chlorpyrifos-oxon [9]. CPF also exhibits non-cholinergic effects such as altering ATP concentrations by altering mitochondria membrane permeability [31].

Carbamates, like methomyl, work through a similar mechanism of action as organophosphates in that they both inhibit the normal function of the acetylcholinesterase

(AChE), however carbamates only inhibit AChE reversibly [32–34]. Inhibition of AChE can mean a buildup of acetylcholine in the neuron synapse causing a repeated signal to the muscles to contract, which ultimately leads to tremors, convulsion, paralysis and death [30,35]. For example, Preventing Agricultural Chemical Exposure (PACE), a large research collaboration focused on Latino and Hispanic men between the ages of 30 and 70 years who are currently farmworkers, includes a total of 235 farmworkers and 212 non-farmworkers working in North Carolina [36]. In the third version of the study (PACE3), farmworkers were recruited and baseline cholinesterase was determined using dried blood samples [37]. Cholinesterase depression was defined as a 15% or more decrease in cholinesterase activity, and over the course of the growing season, cholinesterase depression was at its lowest in June (50.5% of measurements with a 15% depression) to a low in August (14.3% of measurements with a 15% depression) [37]. Farmworkers were on average exposed to between zero and seven pesticides (with measurements above the Limit of Detection) as well [37]. These depressions in cholinesterase are used as a biomarker of carbamate or organophosphate exposure, and these cholinesterase patterns directly align with the Summer growing season in North Carolina.

1.1.4 Pesticide Exposure Assessment.

Using what is excreted from the human body, researchers have developed methods for biologically monitoring pesticide exposure. Arcury et al. (2018) analyzed spot urine samples from PACE participants using high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) [36]. The chemicals included organophosphate insecticides, like the CPF metabolite 3,5,6-trichloropyridinol (TCP), the malathion metabolite malathion dicarboxylic acid, acephate, and methamidaphos; metabolites associated with bis-dithiocarbamate fungicides, such as ethylene thiourea and propylene thiourea ; and metabolites associated with pyrethroid insecticides, such as 3-phenoxybenzoic acid (3PBA), and cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid [36].

When comparing TCP between farmworkers and non-farmworkers, in the 2012 cohort, 67.0% of farmworkers and only 45.7% of non-farmworkers had detectable measurements of TCP [36]. Whereas in the very next year, TCP was detected in all participants, no matter occupational category [36]. The geometric mean of TCP in farmworkers was 3.61 and 3.30 μg/g creatinine in 2012 and 2013, respectively [36]. The non-farmworker exposure concentration geometric means were significantly lower at 2.30 ug/g creatinine in 2012 ($p = 0.003$) [36]. Additionally, the pyrethroid metabolite 3PBA was present in 69.9% of farmworkers and 69.0% of non-farmworkers [36]. Additionally, 3-PBA significantly differed for 3PBA among farmworkers (geometric Mean= 1.04ug/g creatinine, SD= 2.42) and non-farmworkers (geometric Mean= 1.71, SD= 3.43) ($p = 0.004$) [36].

The Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) project is a prospective birth cohort study aimed at quantifying environmental toxicants [38–42]. Women between the ages of 18 and 40 years of age residing in the northern Karst region were recruited between 2010 and 2012 [40]. Participants were pregnant women recruited at 14 weeks of gestation, give or take two weeks [43]. Additional eligibility requirements involved the participants not having used oral contraceptives 3 months prior to pregnancy, *in vitro* fertilization during conception, and did not have any known obstetrics complications associated with their pregnancy. Urine samples were taken at 20 weeks, 24 weeks, and 28 weeks, give or take two weeks [40]. Numerous studies have come out of this cohort that bring information on pesticide exposure and pregnancy that have never been answered before.

In a study on the PROTECT cohort that compares women residing in Puerto Rico pesticide exposure concentrations to the US National Health and Nutrition Examination Survey (NHANES), researchers have found that women residing in northern Puerto Rico had higher mean concentrations of numerous pesticides and their metabolites than NHANES respondents [40]. Specifically, the concentrations of Triclosan and 2,5-Dichlorophenol (25DCP) were two and six-fold greater [40]. In another project from this cohort, researchers quantified pesticide metabolites among 54 pregnant Puerto Rican women [38,43]. For these chemicals, researchers found lower levels of exposure compared to NHANES respondents for each pesticide included [43]. In one organophosphate (malathion dicarboxylic acid) detectable concentrations were 7.7 times more likely to be above the limit of detection if pesticides were ever used inside the home (by the participant or other people living in her home) (95% CI: 2.1, 28.7) [43]. Specifically, when looking at 24DCP detectability, there was a significantly increased association with the consumption of collard greens (OR= 5.9, 95%CI: 1.3, 26.7) and spinach (OR= 4.4, 95% CI: 1.1, 17.9) [38]. These results show that people are exposed to pesticides through diet, household pesticide use, and can be more exposed based on location.

Another exposure study took place in the Jiangsu Province, China, 88 pesticides were investigated for exposure among volunteers at Nanjiang Medical University between the ages of 18.8 and 52 years of age [42]. In total, 76 of the pesticides were found in the blood serum, and of those 58 were found in a human population for the first time [42]. There were large gender differences within this study, specifically, 7 different organochlorines were significantly higher in women than men [42]. For example, Phthalimide was 4.06 times more likely to be detected in women than men, HCH-ß was 46.18 times more likely to be present in women than men, and HCH-Gamma was 3.84 times more likely to be detectable in women than men. There were 5 pesticides with mutagenic properties, 13 pesticides with reproductive toxicity, 3 pesticides with neurotoxicity, and 1 with endocrine disrupting properties [42]. When comparing their results to the literature, people exposed to organochlorines had higher carcinogenic and teratogenic effects on women [42]. This study also compared exposure concentration by the chemical and the region which was also helpful [42]. Out of these chemicals, only chlorothalonil, phthalimide, and propoxur were detected in the US [42].

1.2 Pesticides and Human Health

A review of the literature between 2016 and 2018 found 41% of studies involved neurological disorders, followed by cancer (13%) and poisonings (8%) [44]. Pesticide exposure health harms differ based on the duration, number of exposures, and the concentration of pesticide exposure. In acute pesticide exposures poisoning is a common outcome. For example, a poison center located in Bangkok recorded more than 15,000 patients over a three-year period, 42% of whom had poisonings related to pesticide exposure [45]. The pesticides most associated with these poisonings were insecticides (carbamates, organophosphates, and pyrethroids) [45]. These acute poisonings can also lead to headache, nausea, vomiting, breathing complications, and more symptoms [45]. In another study looking at pesticide poisoning and neurobehavioral function, in farmworkers residing in the People's Republic of China, OP pesticide poisoning accounts for 74.04% of all pesticide poisonings according to the Chinese pesticide registry with the National Institute of Occupational Health and Poison Control at the Chinese Center for Disease Control and Prevention [46].

1.2.1 Cancer and Pesticides.

While acute exposure can lead to poisoning, chronic exposure more often can lead to chronic illness. These pesticides and the associated health harms with them are also often associated with farmwork or residing in a farming area. In a study of over 50,000 farmworkers in the US Agriculture Health Study, the risk ratios of male and female exposed applicators of CPF, an organophosphate (OPP), ranged from 0.72 (95% CI: 0.48, 1.07) for colon cancer to 1.77 (95% CI: 0.70,4.50) or brain cancer [5]. Overall, CPF and OPP exposures have been associated with an increased risk of numerous cancers such as colorectal, esophageal, brain, and lung cancer [2–5].

DDT is also a known carcinogen in humans and commonly still used in LMICs [28,47]. DDT has been linked to increased hepatocellular carcinoma (OR=2.8, 95% CI: 1.1, 7.2) and to reduced prostate specific antigen levels *in vivo* which can cause false negatives [48–51]. Overall, DDT has a clear connection to numerous cancers following rigorous research studies that are epidemiological, *in vivo*, and *in vitro*. DDT, a known environmental estrogen, has also been linked to cancer mechanisms, including breast cancer cell proliferation through G1 to S phase progression by stimulating cyclin synthesis and cyclin-dependent kinase activation [49].

In an investigation of TCP and telomere length alterations, researchers retrieved 1999-2002 data on 1,724 adults age 20 and older[52]. Participants whose exposure concentrations were within the second quartile for urinary TCP concentrations had significantly shorter telomere length (0.06 T/S ratio, 95% CI: 0.02–0.10) in comparison to people in the lowest quartile of exposure[52]. This suggests increased TCP exposure is positively associated with signs of ageing by shortening telomere length, even when controlling for diseases that are strongly associated with telomere shortening like cancer.

While CPF has not been formally evaluated for carcinogenicity by the International Agency for the Research on Cancer, prior research has found organophosphates shorten telomere length and alter LINE-1 DNA methylation suggesting more research was needed in understanding how chlorpyrifos and its metabolites may alter normal cellular function [27,52,53]. Prior studies have attempted to determine a mechanism linking CPF and breast cancer, however there was no clear mechanism since some studies decidedly state this link may be due to estrogenic properties, methylation of H19 gene in organogenesis, or oxidative stress [54–56]. However, an increasing number of studies are linking CPF exposure with breast cancer [3,4,55,57]. *In vitro* and *in vivo*, CPF alters breast cell biology in ways consistent with cancer inducing cell proliferation and oxidative stress [58]. In a 2015 Agriculture Health Study, the spouses of farmers who sprayed CPF were at 1.6 times the risk of developing breast cancer than the wives of farmworkers who did not spray CPF (95% CI: 0.9, 2.9) [4]. Of the women surveyed, only 39% reported ever spraying pesticides themselves suggesting that their primary exposure was purely from whatever comes in on their spouse's clothing[4]. Among wives with a diagnosis of breast cancer, there was a statistically significant association between having spouses who sprayed CPF (HR 1.4, 95% CI: 1.0, 2.0) [4]. There was also an increased risk of premenopausal and postmenopausal breast cancer, HR 1.90 (95% CI: 1.0, 3.8) and HR 0.9 (95% CI: 0.9, 1.9) respectively [4].

In another study, California women who were pregnant between 1959 and 1967 and their daughters (9,300 daughters) were followed for 54 years [51]. These mothers had blood measurements taken of DDT exposure during pregnancy, and the offspring were followed for breast cancer diagnosis by age 52 years [51]. Overall, mothers with the highest o,p-DDT concentrations were 3.7 times more likely to have daughters who developed cancer by the age of 52 in comparison to mothers with the lowest o,p-DDT blood concentrations [51]. Models accounted for possible confounders and effect modifiers like the mothers' weight, lipids, race, age, and breast cancer history which did not explain the relationship [51,59].

1.2.2 Endocrine Disruption and Human Development.

Overall DDT and CPF have been related to hormonal changes within the human body that can effect puberty, mammary developments, and reproduction [28,60]. Using the PROTECT cohort, researchers also studied preterm delivery and other developmental differences due to pesticide exposure in utero [61]. This study aimed to understand how exposure to glyphosate and its metabolite Aminomethylphosphonic acid (AMPA) can affect preterm birth [61]. The research team studied urine samples collected at Visit 1 (18 ± 2 weeks gestation) and Visit 3 (26 ± 2 weeks gestation) and included 53 women whose pregnancy resulted in preterm birth and 194 randomly selected controls [61]. Glyphosate was found in 79.1% and 79.3% of the participants` and AMPA was found in 54.2% and 51.4% of women at Visit 1 and 3, respectively [61]. Preterm birth was significantly associated with glyphosate or AMPA present in urine samples at Visit 3 [61]. Specifically, Adjusted odds ratios of preterm birth were also higher for an interquartile range change of exposure to glyphosate (OR=1.35, 95% CI: 0.99,1.83) and to AMPA (OR=1.67, 95% CI: 1.26, 2.20) at Visit 3 [61].

Organochlorines are endocrine disruptors that mimic estrogen, one of the key hormones regulating ductal morphogenesis. Therefore, previous studies have shown organochlorines, such as DDT, increase cell proliferation in cancer cell lines [49,62]. A myriad of prior research exists suggesting that DDT mimics estradiol and therefore alters normal breast cell growth and development [49,62,63]. DDT has also been shown to transfer across the placenta and breast milk from mother to infant [28]. These results show that pesticide exposure can alter signaling relevant for mammary gland development and carcinogenesis.

In a study quantifying endocrine disruption, mesenchymal stem cells (MSC) were treated with DDT and analyzed via RNA sequencing and multiple phenotypic assays [64]. Untreated MSCs had on average 13.9 colony-forming units (CFUs)/plate, whereas DDT-exposed MSCs had roughly 8.1 CFUs/plate, and DDT treated MSCs had less fibroblast and more spherical phenotypes [64]. The research team suggests this is likely due to a decrease in self-renewal capacity for MSCs exposed to DDT [64]. Additionally, the research team found increased proliferation by day 7 of growth for treated cells [64]. Additionally, researchers found DDT-treated MSCs were associated with an increase in differentiational potential of MSCs [64]. More specifically, MSCs exposed to 1uM DDT for 5 days had a 2.1-fold increase in osteogenic differentiation and 1.8-fold increase in adipogenic differentiation based on high density culturing [64]. Furthermore, when researchers investigated estrogen receptor (ER) positive cells (MCF-7 cell line), they found DDT and ER exposed cells were associated with significantly increased cell proliferation ($p < 0.05$) suggesting DDT has some estrogenic effect [64].

Similarly, a study of the organophosphate CPF exposure and estrogen and progesterone receptor expression in rats. Rats exposed to CPF (1mg/kg/day for 100 days) versus non-exposed rats were associated with a 27% decrease Estrogen Receptor-α detection and a reduction the amount of circulating estradiol and progesterone [65]. Researchers found similar results among pregnant women between the ages of 15 and 36 residing in a rural area of the Rio Negro Province, Argentina, found similar results [66]. In this study, researchers assessed organophosphate exposure among 97 pregnant women [66]. In this area pesticides are sprayed roughly 6 months throughout the year, and blood samples from women looked for organophosphate biomarkers of health like cholinesterase, progesterone and cortisol [66]. From pre- to post-spray season acetylcholinesterase (AChE) was significantly depressed in the average sample from these pregnant women (11% depression, $p \leq 0.01$) [66]. This shows that the women were likely exposed to organophosphates. Additionally, cortisol measurements on average significantly increased 55% after the post-spray season in the region ($p \leq 0.01$) [66]. Based on second trimester data, during the spraying period progesterone concentrations decreased compared to pre-spraying (p=0.07) [66]. The researchers propose these changes in cortisol and progesterone are indicative of impaired hormonal inactivation of progesterone and cortisol, and placental progesterone synthesis across the sample population [66].

1.3 Social Determinants of Health

1.3.1 Farmworkers and Chronic Health Disorders.

Pesticide exposure has been associated with a myriad of neuronal disorders such as Parkinsonism, Alzheimer's, and amyotrophic lateral sclerosis (ALS) [1,44,67–70]. Specifically, in the Parkinson Environment Gene Study (PEG) occupational pesticide exposure and Parkinson's disease were assessed based on self-reported history from workers in central California [67]. In this study, 360 cases and 827 controls were interviewed for work history, family history of Parkinson's Disease, and pesticide use in the past [67]. For people who mixed and loaded the pesticides were 1.62 times more likely to be diagnosed with Parkinson's (95% CI: 1.00-2.60) [67]. Any pesticide use was associated with 2.5 times the risk of developing Parkinson's Disorder (95% CI: 1.50,4.15) [67]. Ultimately carbamates (OR=5.55, 95% CI: 1.81, 17.04), fungicides (OR=3.11, 95% CI: 1.65,5.88), insecticides (OR= 2.10, 95% CI: 1.22, 3.60), herbicides

(OR=2.45 , 95% CI: 1.37, 4.36), and other pesticides such as rodenticides and defoliants (OR= 2.22, 95% CI: 1.11, 4.44) were found to increase risk of Parkinson's Disease [67].

Exposure to pesticides have also been associated with diabetes and cardiovascular disease [1,44,71,72]. A study from the Bang Rakam district of Phitsanulok Province in Thailand collected data on 866 participating people with diabetes mellitus and 1,021 people without diabetes mellitus or a related disorder [71]. Exposure to rodenticides was significantly associated with diabetes mellitus prevalence (OR=1.40, 95% CI: 1.01,1.95) [71]. When testing 35 brand-named pesticides, an organochlorine, organophosphate, fungicide, and carbamate were all found to be significantly associated with an increased likelihood of diabetes mellitus [71].

In a French study, researchers studied insurance billing data for members of The Mutalite Sociale Agricole (MSA) and non-members [73]. Farmworkers were found to have more risk of developing a motor neuron disease (RR=1.13, 95%CI: 0.97,1.31) and Parkinson's Disorder (RR=1.10, 95%CI: 1.02, 1.18) than people who were not members of MSA [73]. In total 9% of MSA members had an incident case of a motor neuron disorder, and of those cases, 70% were farmworkers [73]. For Parkinsonism, 11% of MSA members were diagnosed as an incident case, and of those cases, 74% were farmworkers [73].

1.3.2 Immigrant and Latino Health.

Social demographics like citizenship status makes a large difference in health outcomes for many immigrants residing in the US. Specifically, many Latino and Hispanic immigrants, migrant workers, and the health of farmworkers are often worsened due to systemic prejudice and harmful policies [74]. For example, in a study conducted in Southeast Michigan, 23 clients and 28 service providers from two federal qualified health centers and one non-profit were interviewed to understand how new immigration policies passed during the Trump administration affected immigrants [74]. These health centers where participants were recruited were located in a predominantly Mexican and Central American immigrant area of Detroit, Michigan [74]. More than half of study participants identified as undocumented (N=17) [74].

Based on harmful immigration and enforcement policies created throughout the Trump administration, study respondents reported feeling more isolated within Southeast Michigan and more reliant on the Latino community and health providers [74]. In this qualitative study, immigrants discussed postponing or discontinuing care for fears of deportation, having legal and mental barriers to asking for help from the government or psychologist, and having to rely more on community [74]. One provider even shared how some immigrants during the Trump administration even collaborated to coordinate grocery runs for entire communities to reduce risk of deportation to Latino immigrants residing in Southeast Michigan [74]. The way that many undocumented immigrants are forced into secrecy within the United States causes mental and emotional harm in addition to restricting residents' ability to meet basic needs or access healthcare.

Citizenship status was also a known barrier to health insurance and treatment [75,76]. In a study of 2,702 participants living with diabetes, non-citizens had a greater risk for poor glycemic management (OR=5.16, 95% CI: 3.73, 6.04) in comparison to citizens by birth [76]. Additionally, citizens by naturalization were also at an increased risk of poor glycemic management (OR=1.95, 95% CI: 1.49,2.55) [76]. Additionally, this study found that individuals with diabetes and without health insurance were almost twice as likely to have poor glycemic management compared to insured people (OR=1.99, 95% CI: 1.53-2.59). Similar outcomes have also been noted in cardiovascular disease. Using NHANES, researchers retrieved data from 2011 to 2016 to investigate prevalence, treatment, and control of hypercholesterolemia, included 11,680 US-born citizens, 2,752 foreign born citizens, and 2,554 non-citizens [75]. In this study, over half of non-citizens did not have health insurance (52.2); which was significantly more than US-born citizens (13.6%, $p<0.001$) [75].

Non-citizens also had significantly higher prevalence of diabetes (15.7% vs. 12.8%, $p<0.001$) and [75].Treatment percentages were also significantly lower among non-citizens than US-born citizens with hypercholesteremia (16.4% vs 45.5%), hypertension (60.3% vs. 81.1%), and diabetes (51.2% vs. 69.5%) ($p<0.001$) [75]. Among noncitizens, those without a usual source of health care or health insurance had lower treatment percentages for hypercholesteremia (2.7% and 8.1%), hypertension (22.2% and 39.1%), and diabetes (15.5% and 28.6%) [75]. It is very important to understand that overall, environmental risk factors of the many pesticides on the global market are still poorly characterized across the literature.

1.3.3 Migrant Worker Health.

Migrant workers, people who work seasonally in agriculture and therefore live temporarily from work site to work site, face unique barriers to food, housing, and health care [17,76–85]. The first time we see laws on farmworker housing rights are in 1915 with California's Labor Camp Act in direct response to the Wheatland Hop Riot of 1913 [85]. In the North Carolina Farmworkers Project, researchers at Wake Forest School of Medicine documented migrant worker housing conditions across 16 counties and included over 186 camps [86]. Researchers found roughly two thirds of camps had residents with H-2A visa status, and only over one third of the camps had a certificate of inspection posted (in accordance with the law) [86]. Most camps had a total of 10 to 14 housing violations (n=110, 60.1%), with some having as many as 15 to 22 housing violations (n=26, 14.2%). In farmworker camps that did not have any residents with an H-2A visa, the total violations count was on average 12.7 which was significantly higher than camps that had H-2A visa residents ($p<0.05$) [86].

In a study of two hundred migrant farmworkers recruited in Nebraska were mostly of Mexican descent (n=184, 92.9%), men (n=185, 93.0%), and a mean age of 33.5 years of age [77]. Most of the respondents also had an annual income less than $10,000 (n=110, 61.5%), worked 35 to 50 hours per week (n=110, 61.8%) did not have health insurance (n=135, 71.8%), and no primary care physician (n=157, 83.1%) [77]. In this study researchers use workplace injury as a proxy for workplace safety and found that workers who reported an occupational injury were 7.35 times more likely to also report depression (95% CI: 1.35,39.93) [77].

In countries where agriculture was a major source of income, such as Thailand, most farmworkers (97%) report using pesticides such as organochlorines (OCs) and organophosphates (OPP) [13,87–89]. Two to eight percent of all cancers in low- and middle-income countries (LMICs) are estimated to be due to occupational carcinogen exposure [6]. CPF and OPP exposures have been associated with or increased the risk of numerous cancers such as colorectal, esophageal, brain, and lung cancer [2–5]. Most studies on farmworker pesticide exposure do not include how worker exposure levels compare to the general population's OPP exposure levels, the level of pesticide exposure that was active for cancer-related biological pathways, and the possible mechanisms for primary human breast cells alterations by pesticides.

These barriers to care are found in low- and middle-income countries, like Thailand, as well [90,91]. In a literature review of migrant worker health, an influx of migrant workers from Myanmar, Laos, and Cambodia entered the United States happened in 2010, of which most were undocumented workers [90]. Much of the work that migrant workers complete in Thailand was low paying and not very secure since it was seasonal [90]. Researchers also found that most migrant workers purchased their own medications as opposed to using the healthcare system within Thailand [90].

On top of more commonly developing chronic disorders in comparison to citizen farmworkers, many migrant and non-citizen farmworkers also have more barriers to receiving care. Farmworkers working in the US without citizenship have been associated with increased cardiovascular disease, hypercholesteremia, and diabetes [79]. Moreover, non-citizen farmworkers have also been associated with significantly lower treatment rates of hypertension (60.3% versus 45.5% in citizen farmworkers), hypercholesterermia (16.4% versus 81.1%), and diabetes mellitus (51.2% versus 69.5%) [79]. These differences were also significantly lower when compared to farmworkers who were both US citizens and immigrants (79.6%, 43.3%, and 66.6%) [79]. Overall, migrant workers in agriculture have more barriers to care, and worse health prognosis compared to citizen colleagues. There is more research needed to understand differences in pesticide exposure by social determinants of health like citizenship status and occupation to better understand how pesticides affect human health.

1.4 Dissertation Aims

Overall, migrant workers in agriculture have more barriers to care, and worse health prognoses compared to citizen colleagues based on the current literature. However, more research is needed to understand differences in pesticide exposure by social determinants of health like citizenship status and occupation to better understand how pesticides effect human health. This dissertation expands the current knowledge on pesticide exposure assessment and the effects to farmworker health. In this dissertation, we quantified pesticide exposure to better understand how farmworker exposure differs from the general population. Overall, this dissertation assessed farmworkers' pesticide exposure in northern Thailand; combined publicly available databases, and identified pesticide exposure by social determinants of health and associated mechanisms for cell perturbations. We hypothesized workers have higher levels of pesticide exposure, and more importantly a higher percentage of bioactive pesticide measurements.

The second chapter quantified occupational pesticide exposure levels, focused on OPPs and carbamates, and determined the associated disorder symptomology in a sample of farmworkers residing in norther Thailand. The relationship between workplace air pesticide exposure, health biomarkers and self-reported health concerns in northern Thai farmworkers were also assessed. All farmworkers included in chapter two completed an administered health questionnaire, provided blood and urine samples, and consented to air samples being collected during pesticide spraying. This project adopted a mixed-methods approach during assessment of pesticide exposure and the resulting health effects including: 1) personal air sampling, 2) biomarker sampling, and 3) perceived exposure and health effects assessed by questionnaire.

The cross-sectional study outlined in chapter two quantified exposure to pesticides including chlorpyrifos, methomyl, and metalaxyl, by air sampling and liquid chromatography/mass spectrometry. Chapter two also included multivariate regression analyses to compare serum and urinary biomarker concentrations between farmworkers and comparison workers, adjusting for the potential confounders age, smoking status, alcohol consumption, and body mass index (BMI). We also conducted multivariate linear regression analyses of the association between pesticide levels quantified in the personal air samples and serum and urinary biomarkers in the farmworkers collected after spraying, adjusting for the same covariates as described above. We hypothesized pesticide exposure levels will correlate with decreased white blood cell counts and increased self-reported health concerns related to immune system and nervous system disorders.

Next, chapter three used data from the National Health and Nutrition Examination Study (NHANES) to compare pesticide exposure biomarker concentrations between people with and without a reported history of farmwork and among farmworkers by US citizenship status. NHANES is a cross-sectional study representative of the US population with oversampling weights for minoritized populations. After we stratified by exposure, linear regressions were completed in the sample for each pesticide. The adjusted linear regressions control for urine creatinine or lipid adjustment, body mass index (BMI), age in years, gender, racial ethnicity, education level, poverty-income ratio (representative of socio-economic status), US citizenship, and survey year. To understand how pesticide exposures vary by occupation and citizenship, this study 1) quantified and compared pesticide biomarkers among farmworkers and non-farmworkers, and 2) quantified and compared pesticide biomarkers between citizen and non-citizen farmworkers. We hypothesized that on average farmworkers will have higher concentrations of pesticides biomarkers than non-farmworkers. Furthermore, among farmworkers, we hypothesized non-citizens will have higher pesticide biomarker concentrations than US citizens.

Lastly, in the fourth chapter, NHANES participants' pesticide exposure concentrations were compared to Toxicology Forecaster Dashboard (Toxcast). The US EPA's Toxcast dashboard is a collection of publicly available high throughput toxicity data intended to make chemical assessment more accessible by allowing researchers to search which chemicals are positive or negative for specific assays more efficiently. Toxcast reports the results on thousands of chemicals and assays that are either ‘cell based’ (e.g., cellular viability or proliferation assays) or are ‘cell free’ (e.g. enzyme or protein assays). In this chapter, urinary pesticide biomarker concentrations were compared by social determinants of health such as occupation and US citizenship status. In chapter 4, we labeled anyone who had at least one chemical measurement equal to or above the minimum Toxcast ACC for that chemical as being “bioactive”. Anyone who did not fit this group was defined as “non-bioactive.” Demographics were adjusted for survey weights and quantified by bioactivity status among all study participants. Next, analyses were completed among farmworkers only by US citizenship status.

Next, we calculated bioactivity in chapter 4 by the chemical and marked measurements as bioactive based on their hitcall equaling ‘1’. For model outcomes this bioactivity status by chemical was used as the outcome variable for logistic regression models used to investigate how the odds of being a farmworker and having at least one bioactive measurement differ from non-farmworkers by the chemical. These models were adjusted for BMI, age, poverty-income ratio (PIR), survey year, gender, racial ethnicity, U.S. citizenship status, farmwork history, country of birth and education level. After comparing all study respondents’ odds of having a bioactive measure, we created logistic regression models comparing U.S. citizenship status. In this project, I hypothesized residue and excreted pesticide levels will differ between farmworkers and consumers; with farmworkers from LMICs having higher excreted pesticide biomarker levels in comparison to workers from high income countries.

Chapter 2 Pesticide exposure and adverse health effects associated with farmwork in Northern Thailand

2.1 Introduction

Agriculture is a major contributor to Thailand's economy [92,93]. Among surveyed farmers residing in Northern Thailand, most (97%) reported using pesticides on their crops [13,87–89]. Over 93% of agriculture workers in Thailand work through the informal sector and are not defined as employees under the Labor Protection Act; thus, these workers are exempt from numerous safety laws surrounding labor and social security rights [45,94]. Thailand ranked third out of 15 Asian countries for pesticide use per unit area and fourth in annual pesticide use [93]. Although there have been increases in organic food consumption in Thai markets, there is no evident reduction in pesticide use [93,94]. In fact, pesticide use in Thailand has increased from 110,000 tons in 2007 to roughly 172,000 tons in 2013 [93]. For the pesticides being imported into Thailand, a third are considered extremely, highly, or moderately hazardous based on the World Health Organization's (WHO) hazard categories [93,95].

Pesticide pollution in the environment is associated with poisonings, oxidative stress, neurological dysfunction, birth defects, reproductive disorders, metabolism disorders such as diabetes mellitus, and cancers including colorectal cancer, prostate, and non-Hodgkin lymphoma [1,2,45,71,96,97]. Although Thailand does not have a poisonings registry, a poison center located in Bangkok recorded more than 15,000 patients over a three-year period, 42% of whom had poisonings related to pesticide exposure [45]. The pesticides most associated with these poisonings were insecticides: Carbamates, organophosphates, and pyrethroids [45]. Research is lacking on low- and middle-income countries (LMIC) pesticide exposure and related health outcomes [98].

This study was motivated by a group of contract farmers residing in Wiang Pa Pao, Chiang Rai, Thailand. These farmers reported concerns about their health related to spraying pesticides to Chiang Rai Prachanukroh hospital employees. Our project was launched in response to this concern. The purpose of this project was to assess the pesticide exposure of these farmworkers in Northern Thailand and to understand the resultant health effects when compared to workers residing in a non-agricultural area (Chiang Rai, Thailand). This project adopted a mixed-methods approach during assessment of pesticide exposure and the resulting health effects including: 1) personal air sampling, 2) biomarker sampling, and 3) perceived exposure and health effects assessed by questionnaire.

2.2 Materials and Methods

The STROBE cohort reporting guidelines and checklist were completed in the creation of this dissertation. The study protocol was approved by the Institutional Review Boards of Mae Fah Luang University and the University of Michigan (UM Submission ID: HUM00128091/CR00068767). The local community, village leaders, and healthcare volunteers (laypersons with healthcare training) were imperative to the creation and completion of this research project. These community members were consulted and paid in kind for their expertise in identifying and communicating with stakeholders for the study, coordinating transportation, participant recruitment and engagement, data collection, and translation between English and Thai (2 dialects of Thai included in this study).

2.2.1 Study Population.

All study participants were 21 years of age and older, male, and resided in Northern Thailand. By requesting the support of an international food company, we were able to gain permission to research the farmworkers. All farmworkers were also working as pesticide sprayers either part-time or full-time at the time of the study. Since this study was initiated from the farmworkers, most farmworkers and comparison workers were recruited through word of mouth. Each participant provided oral consent to participate in the study which was noted by the researcher administering the survey in the first step of study participation. Participants were recruited in Chiang Rai, Thailand, using a recruitment script administered (in Thai) by health volunteers—laypersons trained through the Chiang Rai Prachanukroh hospital on patient care and interactions. This script included background information on the study, what to expect as a participant of the study, and more importantly fully defined consent to make it clear to the participants they have the right to stop or deny participation at any point. This recruitment script also included information on compensation and that all participants, no matter their sampling consent, will receive compensation for enrolling and taking the researcher administered survey.

Comparison workers were recruited through word of mouth at Mae Fah Luang University (MFU), with a focus on older males in work fields unrelated to agriculture with no commercial pesticide spraying experience. Farmworkers and comparison workers were recruited between July 1st, 2016 and September 15th, 2016. Overall, there were 97 study participants recruited and examined for eligibility. Twenty-seven comparison workers and seventy farmworkers were retained in the study who met the following inclusion criteria: were 21 years or older, male, residing in Northern Thailand and completed all follow-up.

2.2.2 Sample Collection

All participants completed the consent form by oral confirmation due to literacy differences. Conventional farmworkers consented to allow work observation, active air sampling, and pre- and post-spray urine and blood samples to be taken. Each study participant also completed a researcher-administered survey. Participants received 600 Thai baht (approximately 20 US Dollars) for participation and completion of the study.

We quantified exposure and potential adverse health effects among workers by self-report, biological, and environmental sampling. A 69-item occupational health questionnaire was translated into Thai from English by MFU researchers. The questionnaire was based on a previous study of worker health and occupational noise [99]. The survey was administered to study participants at the local community center and took roughly 35 to 45 minutes to complete. Questionnaire data were collected from July 2017 to the end of August 2017 (conventional farmworkers and comparison workers) and again in January 2018 (sample of comparison workers from Chiang Rai).

Nurses collected 10 mL of urine and 10 mL of blood from workers at the local hospital (Chiang Rai or Wiang Pa Pao). For farmworkers, blood and urine samples were collected within one week prior to pesticide spraying and again within 3 days after pesticide spraying. Whole blood samples were collected in red-top tubes with no anticoagulant, lavender-top tubes with EDTA, and green-top tubes with heparin.

2.2.3 Blood and Urine Analysis.

Urine creatinine, urinary calcium, serum creatinine, serum calcium, and complete blood counts were quantified by Meng Rai Laboratory in Chiang Rai, Thailand. Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) were analyzed using the Ellman method, from whole blood and serum, respectively [100]. AChE was analyzed since it is directly related to AChE inhibition by the pesticides, and BuChE since it can be a sensitive biomarker of exposure to AChE inhibitors [101]. Concentrations of the health biomarkers were compared between conventional farmworkers and comparison study participants, as well as within farmworkers before and after spraying.

2.2.4 Air Sample Collection and Analysis.

Personal pesticide air exposure levels were measured in conventional farmworkers only. GilAir Plus Personal Air Sampling Pumps by Sensidyne, Inc. were calibrated each day with the Gilibrator Calibrator before heading to the field. Air samples were collected during the time farmers were spraying pesticides (14 to 63 minutes) using XAD-2 sorbent tubes (SKC Ltd) based on the standard NIOSH method of 5600 at a flowrate of one liter per minute [102]. Field blanks were collected by opening tubes away from the farms for a similar period of time; no air was drawn through the blanks, but they were otherwise handled identically to actual air samples.

The analysis of pesticide residues was completed by the Lumigen Instrument Center at Wayne State University and researchers were blinded to exposure or worker category. Laboratory controls from a spiked and blank filter were extracted each batch. A calibration check at 10 ng/mL and a solvent blank were run every 10 samples. If a check did not pass within 20% error, the entire 10 sample section was re-run. Calibration curves were prepared the same day per batch by comparing the concentration of and area ratio of analytes to deuterated surrogates. A linear regression was taken, and percent error calculated at each calibration point. If a point did not fall within 20% error of the predicted value, it was dropped from the end of the curve.

Air sampling tubes were extracted as either top portions or bottom portions. Top portions of the tubes contained the retainer ring, filter paper, foam pad, and XAD fill. Bottom portions contained the middle foam pad, and the bottom portion of XAD fill; the bottom foam was discarded. Portions were transferred to a 2 dram vial followed by 1960 µL of methanol and 40 µL of a solution containing 1 µg/mL each of deuterated surrogates. The vials were tightly capped and sonicated for 30 minutes, allowing the bath to heat naturally. Heating was found necessary to achieve equilibration of the surrogates and standards to the XAD fill and improve recoveries. Samples were centrifuged to settle any particulates and 900 µL of sample was diluted with 100 µL of water containing 100 mM ammonium formate, resulting in a final sample containing 10% water and 10 mM ammonium formate. Liquid chromatography mass spectroscopy (LCMS) analysis was completed using a Nexera-X2 ultra performance liquid chromatography (UPLC) with Shimadzu 8040 triple quadrupole mass analyzer. Chromatography was achieved using a Waters acquity UPLC Ethylene-Bridged Hybrid (BEH) C18 (1.2 µm, 2.1 x 50 mm) column eluting with 10 mM ammonium formate in water (mobile phase A) and 10 mM ammonium formate in methanol (mobile phase B).

2.2.5 Worker Observations.

MFU and UM student researchers observed the farmworkers during spraying activities and recorded worker practices for mixing, spraying, and storage of the pesticides on a worker observation form. This included information on the pump used, pump calibration, personal protective equipment (PPE) used, and notes on common practices. Detailed notes on PPE used such as item and material were noted by observers (e.g. gloves made of rubber, latex, or cloth).

Work observations were not completed for comparison workers since they do not perform commercial pesticide spraying.

2.2.6 Statistical Analyses.

For questionnaire data, we calculated summary statistics of demographic data across the entire population. We first tested for crude differences across the measures between the conventional farmers and the comparison workers by t-test for continuous variables or by Fisher's Exact test for categorical variables. Analysis of variance (ANOVA) and multivariate regression analyses were used to compare serum and urinary biomarker concentrations between farmworkers and comparison workers, adjusting for the potential confounders age, smoking status, alcohol consumption, and body mass index (BMI). We also conducted multivariate linear regression analyses of the association between pesticide levels quantified in the personal air samples and serum and urinary biomarkers in the farmworkers collected after spraying, adjusting for the same covariates as described above. Any variables that were not normally distributed were log transformed prior to regression. SAS 9.4 was used to complete regressions and demographic tables, and R 3.5.1 was used to create figures and graphics.

2.2.7 Data Statement.

The de-identified data can be made available via Dropbox upon reasonable request and agreement to a memorandum of understanding of ethics.

2.3 Results

Initially we recruited 73 farmworkers and 29 healthcare workers, and upon further recruitment we were able to secure more general comparison workers through word of mouth recruitment at Mae Fah Luang University. This new general comparison worker group was comprised of 27 men with similar mean age and education backgrounds as the farmworkers. Ultimately, the healthcare workers were overly female and had college educations and were dropped. The 27 comparison workers and the 70 male farmworkers were retained for this study.

Overall, farmworkers and comparison workers had similar demographics, except for BMI, which was significantly lower for farmworkers (Table 1). Farmworkers had a median age of 50 years with a range of 22 to 76 years of age, and comparison workers had a median age of 49 with a range of 39 to 68 years. Most of the workers were married, attended primary and secondary school, drank alcohol 1-3 days per week, and were current smokers (Table 1).

The majority of farmworkers mixed more than once during their pesticide spraying shift (n=43, 68%), and some farmworkers mixed the concentrated pesticides up to 4 times (9.5%). Most of the farmworkers' who wore gloves (n=31, 66%) had on the nitrile gloves provided from our research team, and the second most used glove was chemical resistant (n=9, 19.1%). For farmworkers who wore a hat (n=32), the majority of farmworkers wore a wide brim hat (n=25, 78.1%). None of the farmworkers had access to a respirator, but instead wore cloth face coverings that covered the entirety of their head and neck with only their eyes exposed. Of the 46 farmworkers who wore these face hats, 69.6% were commercially purchased (n=32) and 30.4% were homemade (n=14). Farmworkers protected their eyes using either sunglasses, a face shield, or prescription eyeglasses. Of the farmworkers who covered their clothing, most wore a home-made plastic covering. For those who wore a commercially purchased covering, it was usually a plastic rain poncho. All of the farmworkers re-used their PPE from prior shifts, including gloves, covering/apron, and face hats. Farmworkers were noted drinking water or smoking during mixing, removing one piece of or all PPE to mix the chemicals, mixing chemicals near the eating area and other people, and most commonly washing hands by rinsing them in a natural body of water.

With respect to self-reported health concerns, farmworkers and comparison workers self-reported symptom responses only statistically significantly differed for ‘dizziness’ and ‘shaking or trembling of hands’ (Table 2). Table 3 presents worker self-reported stress levels by worker category based on the Cohen’s Perceived Stress Scale. Overall, comparison workers more frequently reported stress in comparison to farmworkers, although not statistically significantly so. However, when it came to reporting confidence in ability to handle personal problems, only 43% of farmworkers reported feeling confident often in comparison to 78% of comparison workers (p-value = 0.03) (Table 3).

A comparison of cholinesterase activity between the two groups indicated that although there were some farmworkers with higher AChE activity, the AChE levels in farmworkers and comparison workers were not significantly different ($p=0.20$; Figure 1A). Comparison workers had higher BuChE activity levels compared to farmworkers ($p<0.0001$; Figure 1B). AChE and BuChE concentrations were not correlated (Pearson= -0.09, $p=0.35$, 95% CI= -0.29, 0.10). Within farmworkers only, pre-spray and post-spray activity of AChE (0.32, $p=0.01$) and BuChE (0.31, $p=0.01$) were correlated.

Measurements of pesticide residues on air samples are displayed in Figure 2. Overall, ethyl chlorpyrifos and metalaxyl were detected at the highest frequency, while methomyl was less frequently detected. Most chlorantraniliprole (N=60) and methyl-chlorpyrifos (N=61) measurements were below the limit of detection. Ethyl chlorpyrifos, followed by metalaxyl and methomyl, was found in the highest concentrations in the air filter samples.

Table 4 presents linear regression parameter estimates and confidence intervals comparing biomarker concentrations between farmworkers and comparison workers. Eosinophil (p=0.02), urine creatinine (p=0.03), and mean cell volume (MCV) (p=0.04) concentrations were statistically significantly higher in farmworkers than the comparison group. Monocytes (p=0.01), red blood cell counts (p=0.01), white blood cell count (p=0.04), and serum calcium (p=0.02) were statistically significantly lower in farmworkers than comparison workers.

Table 5 presents the unadjusted linear regression beta estimates between natural log of the biomarker concentrations and air sample pesticide content for farmworkers only, and Table 6 presents the adjusted model of the aforementioned relationship. The ratio of pre- and post-spray AChE was significantly lower for increased concentrations of methomyl in air samples (p=0.02). Urinary creatinine and serum calcium were inversely associated with air sample concentrations of metalaxyl.

2.4 Discussion

Pesticide toxicity can be either acute, sub-chronic, or chronic toxicity; with acute being a single large-dose exposure incident, sub-chronic being multiple exposures over weeks or a few months, and chronic toxicity being multiple exposures over several months or years [103]. Different types of toxicity can result in different symptoms and usually the timing of the symptoms are much closer for acute toxicity and for chronic exposures toxicity is usually delayed from exposure [103]. Farmworkers are exposed to pesticides via three pathways: dermal contact, ingestion, or inhalation [103].

Most pesticide research studies include information on farmworker pesticide application at three stages: mixing and loading the pesticides, applying the pesticide spray solution, clean-up of the spraying equipment similar to the study presented in this article [103]. The study presented in this article observed farmworkers at these three stages of pesticide application in accordance with this. However, there are techniques for reducing exposure pathways such as wearing PPE to protect skin, mouth, and eyes; not using power equipment which can reduce aerosolized pesticide; spraying outside to reduced confined space with the pesticide exposure; and storing pesticides in their original packaging [103]. Pesticides exposure can cause blindness, vomiting, or even death [103].

2.4.1 Perceptions of Risk and PPE Use

Our pilot study did not capture data on perceived risk of pesticide spraying and PPE use, but this is something important to consider when looking at use. In a cohort study of cotton farmers in Greece, older growers (mean age of 59.0 ± 3.0 years) were statistically significantly more likely to agree with that harmful pests on their crops were a larger concern than the chemicals used to get rid of them ($t=4.48$, $p \leq 0.01$) when compared younger growers (mean age 27.8 ± 4.9 years) [104]. Additionally, the growers in this study almost never had access to a respirator either, and although still rarely having access to a face mask, goggles or coveralls, younger farmers had significantly greater access than older growers ($t=8.17$, $t=7.05$, $t=5.06$, $p \leq 0.01$) [104]. In a study of pesticide operators in Greece, most of the farmworkers showed unsafe behavior (66.1%) and the majority perceived pesticides as low risk (65.2) [104].

In a study of Iranian farmworker perceptions and PPE use, farmworkers perceived the importance of PPE much higher than the use of PPE and more specifically most of these farmworkers also had little to no respirator use [105]. Many workers ranked the importance of not eating, drinking, or smoking during pesticide application, but some workers still admit to doing so while applying pesticides [105]. In a study on cotton farmers in Northern Greece, PPE use varied depending on the item of clothing with boots and hats being the most common PPE used [106]. While the overwhelming majority of farmworkers reported almost never wearing a respirator, less than 5% reported almost always or occasionally wearing a respirator suggesting that the farmworkers may have access to respirators [106]. In a cohort of Iranian apple farmers, 8% of the 200 farmers reported also preparing pesticides in the kitchen [107]. Overall, apple farmers reported often following safety behaviors like washing hands with soap after spraying (Mean=4.8 out of 5, SD=0.3), not eating or drinking while spraying (Mean=4.8 of out 5, SD=0.4), and not smoking during spraying [107]. Cuenca et al. (2020), studied 257 farmers (44 women and 56 men) in three different communities in Bolivia for PPE use and health outcome concerns of workers [108].

Overall farmers in more tropical regions used organophosphates more than the other communities [108]. A minority (40%) of Bolivian farmers had at least one article of clothing that they could use as PPE, and only 17% of the farmers were well protected with PPE [108]. The majority of farmers (80%) reported feeling sick after spraying pesticides [108]. Additionally, Cuenca et al. (2020) found that headache (80% of women and 70% of men) and dizziness (29% of women and 46% of men) were the most often reported symptoms related to pesticide spraying activities among the farmers [108]. While we did not measure perceptions of risk and PPE use among our farmworkers, since the study was initiated by the farmworkers, it is possible they may perceive pesticide as a higher risk or more harmful than these workers. To better understand perceptions and the needs of the farmworkers, a focus group among these farmworkers should be performed.

Greek farmworkers' perceptions also show the importance of personal safety and safe behavior were not a priority for most workers (with only 44.5% and 41.1%, respectively) [109]. Additionally, in another study in Pakistan on women farmworkers' health, researchers found things like illiteracy, frequency of illness, medical treatment, and traditional treatment were negatively associated with PPE use [110]. In this study, the farmworkers initiated contact with the research team, therefore we hypothesize that these Thai farmworkers may be more concerned with safety and behavior than the farmworkers from these studies. Understanding farmworker perceptions of risk, and how these vary across different cultural and demographic groups, will be essential for designing interventions to encourage PPE use and safe handling of pesticides.

2.4.2 Cholinesterase Depression

Other studies have assessed the relationship between pesticide exposure and cholinesterase activity outside of Thailand. Strelitz et al. measured baseline to post-spraying whole blood and serum cholinesterase levels of 215 farmworkers from the Washington State Cholinesterase Monitoring Program [101]. Ellman colorimetric enzymatic assays were used by two different laboratories to measure BuChE and AChE using the same methodology (Washington State Public Health Laboratories in 2006 and Pathology Associates Medical Laboratories in 2007 to 2011) [101]. In this same study, cholinesterase depression was defined as 20% or greater decrease from baseline to post-exposure cholinesterase exposure [101]. The authors found AChE and BuChE activity to be negatively correlated (-0.14, 95% CI: -0.27, -0.01) [101]. Our study also found the correlation between AChE and BuChE to be weak, but the correlation was non-significantly positive (0.05, 95% CI: -0.20, 0.29). The ratio of pre-and post- spray AChE activity was also significant lower with increasing concentrations of methomyl in air samples. Others have reported BuChE activity as a more sensitive biomarker of exposure to cholinesterase inhibitors than AChE [101,111,112]. In our study, BuChE activity was significantly lower in conventional farmworkers relative to comparison workers.

A cross-sectional study in greenhouse and packinghouse workers residing in Ethiopia also used the Ellman method to analyze serum cholinesterase. In total, this study looked at 588 flower farm workers [113]. This study included 311 women who work in the greenhouse (n=156) or the packinghouse (n=155) [113]. When completing a t-test between sprayers and non-sprayers, there was not a significant difference in BuChE outcomes (25.9 vs 24.2, p=0.85) [113]. Most of their farmworkers used a half face respirator mask, gloves, boots and overalls (with one farm having textile only and not chemical proof overalls [113]. The chemicals sprayed by the sprayers were predominantly un-registered chemicals (n=45, 29.2%) [113]. These farmworkers sprayed numerous types of pesticides but there was some overlap with our study since they used organophosphates (n=10,8.9% of all pesticides used) [113].

Researchers in SE Iran completed a case control study of 141 family members of farmworkers focused on organophosphate and organic chlorine pesticides [114]. It was found AChE activity was significantly decreased compared to control subject (p<0.001) [114]. This study also found an inverse relationship with paraoxonase 1, superoxide dismutase, glutathione peroxidase, and total antioxidant capacity amount which suggest epigenetic change and oxidative stress among farmworkers [114].

In a study by Sapbamrer and Nata (2014), rice farmers and non-farmer controls residing in Northern Thailand were interviewed and had blood samples taken to ascertain their overall pesticide exposure [115]. When looking at self-reported health outcomes in our study, farmworkers did not have differences in respiratory symptoms relative to comparison workers. Farmworkers reported trembling in their hands less often in comparison to other workers, however with exposure to AChE inhibitors, we would expect farmworkers would report trembling more often.

2.4.3 Health Biomarkers and Symptoms

When trying to assess cholinesterase changes due to chemical exposure, it is standard to take pre- and post-spraying samples to ascertain measurement alterations before and after exposure to pesticides [101,116–120]. The Washington Cholinesterase Monitoring Program takes one baseline sample prior to spraying and one follow up sample taken at one or more follow-up visits after a suspected exposure to organophosphates [101,120]. In Quandt et al. (2010), researchers assessed the health of 231 migrant farmworkers (H-2A US Visa) residing across 11 counties in North Carolina took four blood collections to determine cholinesterase depression [37]. In the Australian Cholinesterase Research Outreach Project (CROP), researchers analyzed cholinesterase depression among farmworkers and non-farmworkers residing in South West Victoria took one baseline sample and three subsequent samples at different times thought to be high post-exposure times [117].

Among cotton farmers in Pakistan, 82% of farmers reported a health impairment with the most common symptoms being irritation of skin and eyes, headache and dizziness [121]. Farmers had none of the aforementioned symptoms within 24 hours prior to spraying pesticides and on average reported approximately three different symptoms (2.6 ± 0.88) [121]. Studies that also looked at DNA damage and liver function biomarkers, found farmers in Pakistan were exposed to 5 different chemicals including chlorpyrifos-methyl, carbosulfan, profenofos, cypermethrin, endosulfan sulfate [118]. In a study that looks at serum cholinesterase as a function of liver function, found slightly lower serum cholinesterase levels compared to non-industry/pesticide workers (69.2% vs. 19.2%, respectively) [118]. Garcia-Garcia et al. 2015 completed a similar study comparing 169 green house workers who were exposed to pesticides and controls who were unexposed to pesticides in southeastern Spain [122]. Among this cohort, researchers controlled for sex, age, and BMI, but not smoking status. They found BuChE inhibition, RBC, MCV, and neutrophil levels significantly increased, whereas eosinophils significantly decreased and monocytes were not different [122].

Our study assessed hematological parameters by measuring differences in complete blood counts between farmworkers and comparison workers, while controlling for BMI, age, former smoker status, current smoker status, worker category, and pesticide exposure levels. Serum calcium was statistically significant by both worker category and was also significantly different in farmworker. MCV, monocytes, RBC, eosinophils, urine creatinine and WBC were significantly different between our Northern Thailand farmworkers and comparison workers. In our study, farmworkers had a reduction, in RBC and neutrophils, whereas MCV increased (opposite of Garcia-Garcia et al.). Monocytes and eosinophils reduced and increased, respectively in our study and the Garcia-Garcia et al. study [122]. In our study, an association existed between pesticide exposure and all the aforementioned blood counts when looking at farmworkers and comparison workers.

Finally, a cohort study on farmworkers in China before and after pesticide spraying points to the effects of pesticides on complete blood count (CBC) having an effect that can be categorized as long term (3 years) or short term (10 days or less) [123]. Long term results include decreased white blood cell count [123]. Short term results include alterations in complete blood count, hepatic and renal functions, nerve conduction velocities, and on monocytes, hemoglobin and platelet counts [123]. When comparing air sample measurements of methomyl, chlorpyrifos and metalaxyl, we did not identify differences in blood count analytes by chemical exposure, although we identified a range of differences when comparing farmworkers and comparison workers. Prior research has reported significant changes to hematological biochemistry as a result of pesticide exposures causing oxidative stress [122]. Oxidative stress due to pesticide spraying has also been related to changes in CBC such as monocyotsis, leukocytosis, neutrophilia, and lymphocytopenia which were thought to also be closely related to patients in oxidative stress [122,124].

2.4.4 Strengths and Limitations.

Our study has some limitations. We focused on farmworkers who contracted to numerous farms, and therefore workers were likely exposed to other chemicals that were not quantified in this study. These farmworkers may have been exposed to chemicals that were not captured by this study because they sprayed pesticides on other farms and sprayed other plants on the same farms. Due to this discrepancy, our farmworkers' baseline cholinesterase measurements may not actually represent a true baseline measurement due to their overlapping work schedules. The US Occupational Safety and Health Administration (OSHA) generally recommends testing for baseline cholinesterase levels after not working with organophosphates for at least 30 days [125]. OSHA also recommends taking a baseline measurement before and after organophosphate use (with at least a 30 day washout period for both) [125]. This pilot study did not take multiple baseline measurements, and the one baseline that was taken was likely taken before the OSHA recommended guideline of 30 days since pesticide use. However, due to the crop production schedule and growing seasons in Northern Thailand, this population does not appear to ever experience thirty days of no pesticide use. Our study also focused on workplace sampling at a time when the specific farm of interest was expected to be spraying chlorpyrifos, therefore the study results show an over-representation of chlorpyrifos. Additionally, by using an administered questionnaire to capture information from the farmworkers and non-farmworkers, it is possible to have an increase in recall bias, especially for people with long work histories. We did not ask about pesticide use in the home and this could also have been something interesting to ask, especially since it is possible that this is information many respondents may not think of when considering pesticide exposure or use. The potential also exists that farmworkers may not have felt comfortable revealing adverse health outcomes related to their occupation.

Overall, this is the first study of its type that took a mixed-methods approach using survey, biomarker, and workplace observation data to analyze farmworker pesticide health effects in comparison to other workers in Northern Thailand. Additionally, this pilot study is one of the larger studies on farmworker chemical exposures in Thailand. These data can inform the methods for future global occupational health research on farmworkers. Work observations also included a more detailed outline of PPE used by the farmworkers and could inform future studies on PPE in relation to pesticide exposure and preventive interventions. This study will contribute to the building literature on farmworker pesticide exposure and resultant health outcomes.

2.5 Figures

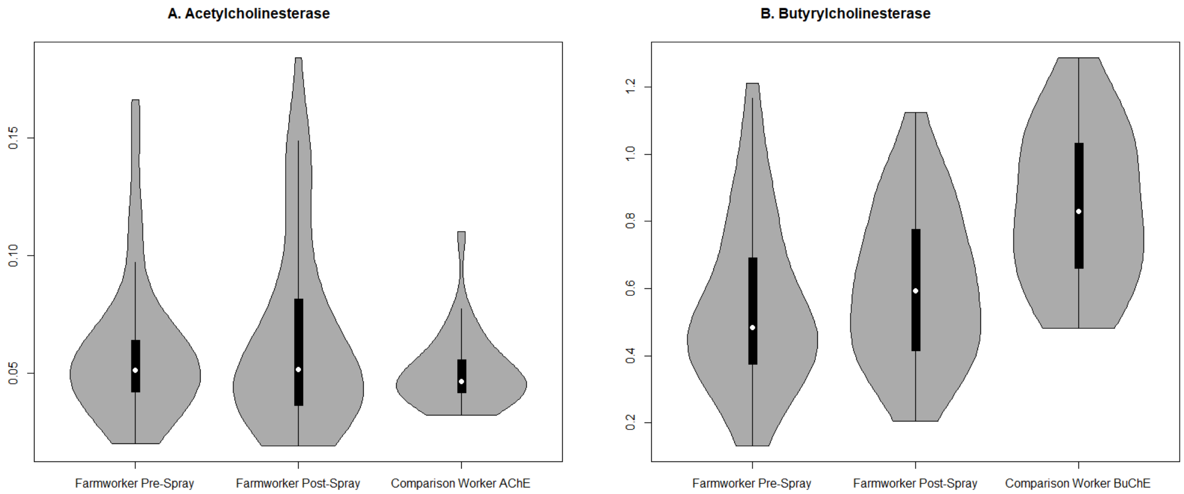
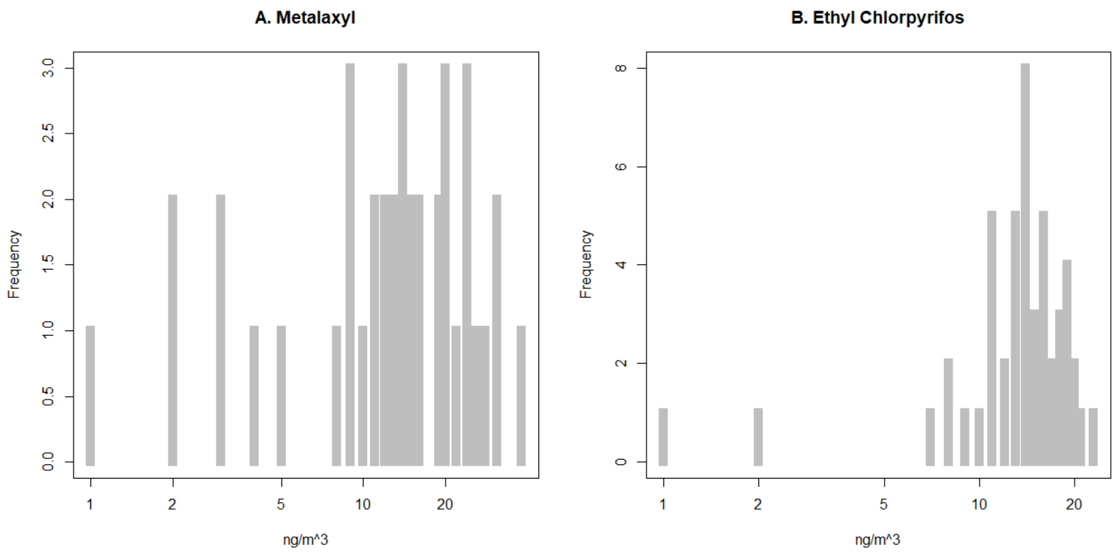


Figure 1 Distribution of acetyl- and butyrylcholinesterase activities in comparison workers (one time point only) and farmworkers (pre- and post-spray)

In previous research completed in Northern Thailand, the AChE national reference range is 6,400 U/L to 8,200 U/L[36 115]. N=97.



Pesticides[a]	LOD (ppb)	<LOD (N)	Minimum	1st Quantile	Median	Mean	3rd Quantile	Maximum
Methyl Chlorpyrifos	0.31	51	--	--	--	--	--	--
Chlorantraniliprole	0.25	51	--	--	--	--	--	--
Methomyl	0.28	44	8.4	9.7	12.9	469.2	21.5	13,483.9
Metalaxyl	0.44	11	13.9	359.2	1,063.6	2,797.6	2,613.2	41,191.3
Ethyl Chlorpyrifos	0.33	3	11.6	4,403.5	8,379.1	12,670.1	14,500.2	67,759.8

Figure 2 Test Pesticide measurements captured by personal air monitors on Northern Thailand farmworkers (N=51) with histograms showing distributions of detected values of (A) metalaxyl and (B) ethyl chlorpyrifos

The units of these measurements are ng/m^3.
Measurements below the limit of detection (LOD) have been removed from the histograms since they have falsified values due to calculations.
Measurements below the LOD have been removed from the histograms and the values for the calculated ng of pesticide per cube meter of air (calculation: [(filter*2) / ((calibration of liters per minute)*minutes of spraying)(1/1000)])

2.6 Tables

Table 1 Study Participant Demographics, among men only

Variable	Variable Outcome	Comparison Worker		Farmworker		Fisher's Exact Significance
		N=27	%	N=70	%	p-value
Marital Status	single	6	22.2	7	10.0	0.11
	married	16	59.3	55	78.6	
	divorced	3	11.1	2	2.9	
	living with partner	2	7.4	6	8.6	
Education	none	0	--	3	4.3	0.48
	primary	18	66.7	49	70.0	
	secondary	6	22.2	15	21.4	
	some college	1	3.7	2	2.9	
	4 year degree	1	3.7	1	1.4	
	graduate Level	1	3.7	0	--	
Alcohol	never	7	26.9	12	17.4	0.64
	not much	4	15.4	14	20.3	
	1-3 days per week	9	34.6	18	26.1	
	4-6 days per week	4	15.4	14	20.3	
	daily	2	7.7	11	15.9	
Tobacco Use	never smoked	8	30.8	25	35.7	0.67
	former smoker	8	30.8	15	21.4	
	currently smokes	10	38.5	30	42.9	
		Median	STD	Min.	Max.	
Comparison Worker Age		49.0	8.1	39.0	68.0	0.61
Farmworker Age		50.0	11.0	22.0	76.0	
Comparison Worker BMI		24.7	3.7	20.0	34.1	0.02[a]
Farmworker BMI		22.2	3.5	16.1	33.1	

[a]Signifies a p-value less than 0.05

Table 2 Northern Thailand Participant Self-reported Symptoms by worker category

	Variable Outcome	Comparison Worker		Farmworker		Fisher's Exact Test
		N=27	%	N=70	%	p-value
In the last two weeks, how often have you had the following conditions…						
stuffy, itchy, runny nose?	rarely or never	18	66.7	51	72.9	0.31
	occasionally	8	29.6	15	21.4	
	frequently	--	--	2	2.9	
watery, itchy eyes?	rarely or never	20	74.1	47	67.1	0.12
	occasionally	4	14.8	20	28.6	
	frequently	2	7.4	2	2.9	
sinusitis or sinus problems?	rarely or never	25	92.6	68	97.1	0.19
	occasionally	1	3.7	---	--	
	frequently	--	--	1	1.4	
pneumonia?	rarely or never	26	96.3	62	88.6	0.14
	occasionally	--	--	5	7.1	
	frequently	--	--	3	4.3	
dizziness?	rarely or never	23	85.2	45	64.3	0.03
	occasionally	3	11.1	23	32.9	
	frequently	--	--	1	1.4	
nausea/vomiting?	rarely or never	25	92.6	61	87.1	0.2
	occasionally	1	3.7	8	11.4	
	frequently	--	--	--	--	
Being absentminded, forgetful, or confused?	rarely or never	12	44.4	31	44.3	0.55
	occasionally	11	40.7	29	41.4	
	frequently	3	11.1	10	14.3	
headache?	rarely or never	17	63.0	48	68.6	0.4
	occasionally	9	33.3	20	28.6	
	frequently	--	--	1	1.4	
loss of appetite?	rarely or never	23	85.2	62	88.6	0.38
	occasionally	3	11.1	7	10.0	
	frequently	--	--	--	--	
fast heart rate?	rarely or never	25	92.6	57	81.4	0.14
	occasionally	1	3.7	10	14.3	
	frequently	--	--	1	1.4	
difficulty with balance?	rarely or never	21	77.8	59	84.3	0.24
	occasionally	5	18.5	10	14.3	
	frequently	--	--	--	--	
blurred vision or double vision?	rarely or never	16	59.3	41	58.6	0.15

	occasionally	9	33.3	25	35.7	
	frequently	--	--	3	4.3	
numbness or pins-and-needles in your hands or feet?	rarely or never	15	55.6	41	58.6	0.32
	occasionally	11	40.7	25	35.7	
	frequently	--	--	4	5.7	
shaking or trembling of your hands?	rarely or never	18	66.7	61	87.1	0.01
	occasionally	8	29.6	6	8.6	
	frequently	--	--	1	1.4	
twitches, jerks, or involuntary movements of your arms or legs?	rarely or never	20	74.1	54	77.1	0.39
	occasionally	6	22.2	12	17.1	
	frequently	--	--	1	1.4	

Table 3 Northern Thailand Participant Stressor Responses, by worker category

Variable	Variable Outcome	Comparison Worker		Farmworker		Fisher's Exact Test
		N=27	%	N=70	%	p-value
In the last month, how often have you felt that you were unable to control the important things in your life?	never	17.0	63.0	48.0	68.6	0.57
	almost never	4.0	14.8	13.0	18.6	
	sometimes	5.0	18.5	8.0	11.4	
	fairly often	1.0	3.7	1.0	1.4	
	Often	--	--	--	--	
In the last month, how often have you felt confident about your ability to handle your personal problems?	never	--	--	4.0	5.7	0.03
	almost never	--	--	2.0	2.9	
	sometimes	2.0	7.4	6.0	8.6	
	fairly often	4.0	14.8	28.0	40.0	
	Often	21.0	77.8	30.0	42.9	
In the last month, how often have you felt that things were going your way?	never	--	--	--	--	0.27
	almost never	--	--	1.0	1.4	
	sometimes	8.0	29.6	28.0	40.0	
	fairly often	6.0	22.2	22.0	31.4	
	Often	13.0	48.2	19.0	27.1	
In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	never	16.0	59.3	49.0	70.0	0.34
	almost never	8.0	29.6	11.0	15.7	
	sometimes	3.0	11.1	10.0	14.3	
	fairly often	--	--	--	--	
	Often	--	--	--	--	
	range	median		standard deviation		
How many hours do you work in a typical week in total at all of your jobs?	6-140	50.96		25.73		

Table 4 Linear regression analyses comparing biomarker concentrations in farmworkers and comparison workers

Analytes	Units	Farmworkers=1 vs. Comparison=0 N=97							
		Unadjusted Values				Adjusted Values			
		β Estimate	p-value	95% CI		β Estimate[b]	p-value	95% CI	
log(Basophil)	%	0.08	0.25	0.23	-0.06	0.12	0.13	-0.04	0.29
log(Eosinophil)	%	0.19	0.05	0.00	0.37	0.24[a]	0.02	0.04	0.44
log(Hemoglobin)	g/dl	-0.02	0.06	-0.03	0.00	-0.01	0.16	-0.03	0.01
log(Hematocrit)	%	-0.01	0.15	-0.03	0.00	-0.01	0.28	-0.03	0.01
log(Lymphocyte)	%	-0.04	0.25	-0.10	0.03	-0.03	0.41	-0.10	0.04
log(Mean corpuscular hemoglobin concentration (MCHC))	pg	0.00	0.37	-0.01	0.00	<0.01	0.53	-0.01	0.01
log(Mean Corpuscular Volume)	fl	0.03	0.02	0.00	0.06	0.03 [a]	0.04	<0.01	0.06
Monocyte	%	-1.09	0.02	-1.99	-0.20	-1.33 [a]	0.01	-2.30	-0.37
Neutrophil	%	-0.48	0.87	-6.49	5.53	-1.34	0.69	-8.08	5.39
log(Plate Count)	cells/uL	-0.02	0.52	-0.09	0.04	-0.03	0.39	-0.10	0.04
log(Red Blood Cell (RBC) Count)	cells/uL	-0.04	0.00	-0.08	-0.01	-0.04 [a]	0.01	-0.07	-0.01
log(RBC Distribution Width (RDW))	%	0.01	0.15	-0.01	0.03	0.01	0.19	-0.01	0.03
log(White Blood Cell Count)	cells/uL	-0.07	0.01	-0.12	-0.02	-0.06 [a]	0.04	-0.12	<0.01
Serum Calcium	mg/dl	-0.83	0.00	-1.31	-0.35	-0.79 [a]	<0.01	-1.31	-0.27
log(Serum Creatinine)	mg/dl	-0.04	0.04	-0.08	0.00	-0.03	0.22	-0.07	0.02
log(Urine Calcium)	mg/dl	0.13	0.12	-0.03	0.30	0.14	0.14	-0.05	0.32
Urine Creatinine	mg/dl	33.03	0.03	3.82	62.24	36.29 [a]	0.03	4.17	68.41
log(Acetylcholinesterase)	U/L	0.08	0.37	-0.10	0.27	0.05	0.64	-0.16	0.25
log(Butyrylcholinesterase)	U/L	-0.48	<0.01	-0.68	-0.29	-0.50a	<0.01	-0.70	-0.29

[a]Signifies a p-value less than 0.05

[b]These values were adjusted for age, former smoker status, current smoking status, alcohol use, and BMI.

Table 5 Unadjusted Linear regression models of association between blood analytes and pesticide air sample concentrations

Analytes	Units	Log(Methomyl)				Log(Metalaxyl)				Log(Ethyl Chlorpyrifos)			
		β	p-value	95% CI		β	p-value	95% CI		β	p-value	95% CI	
log(Basophil)	%	0.18	0.68	-0.70	1.06	-0.05	0.52	-0.22	0.12	0.03	0.67	-0.13	0.20
log(Eosinophil)	%	0.18	0.72	-1.14	0.79	0.00	0.96	-0.19	0.18	-0.02	0.82	-0.20	0.16
log(Hemoglobin)	g/dl	-0.01	0.70	-0.09	0.06	0.01	0.38	-0.01	0.02	0.01	0.18	0.00	0.02
log(Hematocrit)	%	-0.04	0.30	-0.12	0.04	0.01	0.43	-0.01	0.02	0.01	0.47	-0.01	0.02
log(Lymphocyte)	%	0.12	0.48	-0.22	0.46	-0.01	0.68	-0.08	0.05	-0.05	0.12	-0.11	0.01
log(MCHC)	pg	0.03	0.25	-0.02	0.08	0.00	0.97	-0.01	0.01	0.00	0.47	-0.01	0.01
log(MCV)	fl	-0.06	0.40	-0.20	0.08	0.02	0.16	-0.01	0.05	0.02	0.24	-0.01	0.04
Monocyte	%	-1.54	0.48	-5.91	2.83	0.06	0.88	-0.79	0.92	-0.18	0.66	-1.00	0.64
Neutrophil	%	0.98	0.95	-31.15	33.12	1.51	0.62	-4.71	7.73	3.90	0.18	-1.88	9.68
log(Plate Count)	cells/uL	0.05	0.72	-0.22	0.32	-0.03	0.29	-0.08	0.02	-0.05	0.02	-0.10	-0.01
log(RBC)	cells/uL	0.02	0.82	-0.14	0.17	-0.01	0.39	-0.04	0.02	-0.01	0.49	-0.04	0.02
log(RDW)	%	0.05	0.26	-0.04	0.13	0.00	0.74	-0.02	0.01	0.00	0.62	-0.02	0.01
log(WBC)	cells/uL	-0.03	0.82	-0.27	0.22	0.00	0.93	-0.05	0.05	-0.01	0.80	-0.05	0.04
Serum Calcium	mg/dl	0.08	0.73	-0.39	0.55	-0.40	0.07	-0.83	0.03	0.15	0.53	-0.33	0.62
log(Serum Creatinine)	mg/dl	-0.02	0.18	-0.06	0.01	-0.01	0.44	-0.05	0.02	0.02	0.33	-0.02	0.05
log(Urine Calcium)	mg/dl	0.02	0.78	-0.12	0.16	-0.02	0.73	-0.15	0.11	0.09	0.21	-0.05	0.22
Urine Creatinine	mg/dl	5.17	0.67	-19.35	29.69	-18.99	0.09	-41.37	3.39	9.23	0.45	-15.36	33.83
AChE Ratio[b]	U/L	-0.31	0.05	-0.62	-0.01	0.06	0.71	-0.24	0.35	0.16	0.31	-0.16	0.48
BuChE Ratio[b]	U/L	-0.06	0.62	-0.33	0.20	-0.06	0.63	-0.30	0.19	-0.15	0.26	-0.41	0.11

[a]Signifies a p-value less than 0.05

[b]Cholinesterase ratios were calculated by dividing post measures by pre measure.

Table 6 Adjusted Linear regression models of association between blood analytes and pesticide air sample concentrations (N=51)

Analytes	Units	Log(Methomyl)[c]				Log(Metalaxyl)[c]				Log(Ethyl Chlorpyrifos)[c]			
		β	p-value	95% CI		β	p-value	95% CI		β	p-value	95% CI	
log(Basophil)	%	0.28	0.52	-0.61	1.16	-0.19	0.05	-0.39	0.00	-0.03	0.83	-0.30	0.25
log(Eosinophil)	%	-0.25	0.64	-1.33	0.84	-0.07	0.56	-0.33	0.19	-0.22	0.17	-0.54	0.10
log(Hemoglobin)	g/dl	-0.01	0.79	-0.09	0.07	-0.01	0.41	-0.03	0.01	-0.01	0.40	-0.03	0.01
log(Hematocrit)	%	-0.02	0.65	-0.11	0.07	0.00	0.80	-0.02	0.01	-0.01	0.38	-0.04	0.02
log(Lymphocyte)	%	0.18	0.33	-0.19	0.54	0.00	0.94	-0.09	0.09	-0.08	0.15	-0.19	0.03
log(MCHC)	pg	0.01	0.68	-0.04	0.06	-0.01	0.37	-0.02	0.01	0.00	0.83	-0.01	0.02
log(MCV)	fl	-0.07	0.28	-0.22	0.07	0.02	0.27	-0.02	0.05	0.01	0.50	-0.03	0.06
Monocyte	%	-1.38	0.58	-6.49	3.73	0.32	0.59	-0.91	1.55	0.18	0.82	-1.40	1.76
Neutrophil	%	-1.61	0.93	-38.75	35.53	0.93	0.83	-7.98	9.83	6.35	0.24	-4.69	17.40
log(Plate Count)	cells/uL	0.13	0.37	-0.16	0.41	0.00	0.98	-0.07	0.07	-0.07	0.12	-0.15	0.02
log(RBC)	cells/uL	0.05	0.49	-0.11	0.22	-0.02	0.26	-0.06	0.02	-0.03	0.27	-0.07	0.02
log(RDW)	%	0.05	0.26	-0.04	0.14	0.00	0.86	-0.02	0.03	0.00	0.93	-0.03	0.03
log(WBC)	cells/uL	-0.12	0.31	-0.37	0.12	0.01	0.83	-0.05	0.07	-0.02	0.62	-0.10	0.06
Serum Calcium	mg/dl	0.13	0.62	-0.38	0.63	-0.55[a]	0.02	-1.02	-0.08	0.18	0.59	-0.48	0.83
log(Serum Creatinine)	mg/dl	-0.02	0.20	-0.06	0.01	-0.01	0.55	-0.05	0.03	0.03	0.22	-0.02	0.08
log(Urine Calcium)	mg/dl	0.02	0.81	-0.13	0.17	-0.05	0.52	-0.19	0.10	0.07	0.45	-0.12	0.26
Urine Creatinine	mg/dl	6.39	0.64	-20.60	33.38	-30.17[a]	0.02	-54.94	-5.41	5.70	0.74	-29.21	40.61
AChE Ratio[b]	U/L	-0.37[a]	0.02	-0.66	-0.07	0.05	0.72	-0.25	0.36	0.35	0.08	-0.04	0.74
BuChE Ratio[b]	U/L	-0.13	0.35	-0.40	0.15	-0.05	0.70	-0.33	0.22	-0.12	0.50	-0.48	0.24

[a]Signifies a p-value less than 0.05

[b]Cholinesterase ratios were calculated by dividing post measures by pre measures.

[c]These values were adjusted for age, former smoker status, current smoking status, alcohol use, and BMI.

Chapter 3 Assessing Pesticide Exposure among Farmworkers by US Citizenship Status

3.1 Introduction

Pesticide exposure has been linked to a myriad of human health outcomes such as obesity, immune alteration, cancer, neurological conditions, type II diabetes mellitus, and death [7,10,126,127]. More specifically, many pesticides are strong endocrine disruptors because they mimic hormones that the body recognizes and often needs like estrogens and androgens [7,128,129]. Exposure to pesticides can be acute or chronic. Acute pesticide exposure usually refers to a large dose at a one timepoint or over a short duration of time such as five hours or less [128]. Acute pesticide exposure is often associated with vomiting, nausea, tremors, and even death. More recent toxicologic research also finds acute exposure can also disrupt the endocrine system and cause alterations to cell proliferation based on the cell type [65,128,130–132]. Chronic exposure encompasses multiple exposures over time and are commonly more frequent exposures to lower concentrations of pesticides [133]. Understanding and studying the health effects of chronic exposure is difficult because the outcomes are often chronic health conditions like diabetes, cancer, or cardiovascular disease which may take years to develop [79,134–137].

Comparing acute and chronic exposure is further complicated by pesticides that persist in the environment. Non-persistent pesticides (NPPs) do not last in the environment or human body for more than a matter of hours or days. NPPs include chemicals like organophosphates, chlorinated phenols, carbamates, pyrethroids, toluene, and fungicides [133]. Persistent organic pollutants (POPs) are chemicals that do not degrade in the environment for a very long time such as years or decades[133]. POP pesticides include organochlorines, lindane, chlordane, and dieldrin[133]. Persistent pesticides like the organochlorine dichlorodiphenyltrichloroethane (DDT) are the focus of numerous superfund sites, due to the toxicity and longevity of these chemicals in the environment [7].

Additionally, social determinants of health like occupation or citizenship can alter both exposure and health outcomes. Healthcare policy and services are limited to non-existent for immigrants and especially migrant workers residing in the US. Many policies that on the surface appear highly beneficial for the American people like the Affordable Care Act of 2010, actually exclude immigrants completely from accessing care[81]. In addition, agreements like the North American Free Trade Agreement between the US, Canada, and Mexico limit migrant worker rights[17]. Moreover, migrant worker health is often unprotected by the law and workplace discrimination leaves migrant workers very vulnerable [77,81,138,139]. Prior research on migrant workers in the US Midwest found factors like economics, logistics, and health significantly affected the mental health of migrant workers [140].

Additionally, there is little to no literature on self-reported US citizenship in NHANES and pesticide exposure, especially among farmworkers. To address these gaps and understand how pesticide exposures vary by occupation and citizenship, this study aims to 1) quantify and compare pesticide biomarkers among farmworkers and non-farmworkers, and we go further to 2) quantify and compare pesticide biomarkers between citizen and non-citizen farmworkers. We hypothesize that on average farmworkers will have higher concentrations of pesticides biomarkers than non-farmworkers. Furthermore, among farmworkers, we hypothesize that non-citizens will have higher pesticide biomarker concentrations than US citizens.

3.2 Methods

3.2.1 The National Health and Nutrition Examination Survey (NHANES)

NHANES is a cross-sectional study representative of the US population with oversampling weights for minoritized populations. NHANES is a longitudinal assessment of the health and nutrition of adults and children residing within the US. The current iteration of the study began in 1999. Study participants are enrolled on a continuous basis, with data analyzed and deposited in two-year windows. NHANES collects extensive information on the study participants such as self-reported occupation, urinary and serum biomarkers, and self-reported demographics such as age, gender, citizenship, poverty-income ratio, and education.

3.2.2 Study Population

This study included NHANES study participants age 18 years and older who also had occupation and pesticide exposure data present between 1999 and 2014. This study integrated 29 datasets from NHANES laboratory data to understand pesticide exposure, occupation, and demographics of the study population. From the Industry and Occupation Survey, individuals were coded as "farmworker" or "non-farmworker" using the Current Industry (OCD230=1,OCD231=1), Current Occupation (OCD240=18, OCD241=18), Longest Industry (OCD390=1,OCD391=1), and Longest Occupation (OCD392=18), where all participants who put "Agriculture, Forestry and Fishing" were coded as a farmworker.

From the demographics data, DMDEDUC2 (older than 18 years of age) and DMDEDUC3 (l8 years of age and younger) were combined to create one education level based on the DMDEDUC2 categories. The US citizenship variable (DMDCITZN) is defined as 1= "Citizen by Birth or naturalization" and 2= "Not a citizen of the US", and we removed anyone who responded with "Refused", "Don't Know", or skipped the question.

3.2.3 Biomonitoring Samples and Detectability

NHANES performs chemical biomonitoring in respondents by collecting urine and blood serum collection. Respondents provided their urine by collecting a partial void in a sterile sampling cup at the mobile examination center. Blood samples are collected by certified laboratory professionals. Urine and blood samples are then analyzed for chemical metabolites using isotope dilution gas chromatography high-resolution mass spectrometry (GC/IDHRMS). Pesticide biomarkers measured in blood samples and reported as either 1) fresh weight basis (i.e., pg/g serum) and 2) lipid weight basis (i.e., ng/g lipid). The lipid adjusted values account for blood lipid concentrations and are of particular importance for the accurate quantification of lipophilic pesticides.

All chemical analyses have a limit of detection (LOD) based on the minimum concentration of the chemical that can be accurately measured by the analytical method. Values for a chemical biomarker with measurements below the LOD in are imputed as $LOD/\sqrt{2}$. Detectability, defined as whether the chemical is detected above the LOD for a given individual, was determined using the comment code for each chemical measurement to determine if it was above or below the LOD. Detectability percentages for a given chemical across all participants in which that chemical was measured were calculated by dividing the total number of measurements above LOD by the total number of the chemical's measurements in NHANES.

Using the comment code on each chemical measurement in NHANES to determine if a measurement was above or at and below LOD, we quantified detectability rates for all participants retrieved from NHANES. These detectability rates were first tested by year and by chemical, then detectability was compared by farmwork category. Once stratified by farmwork category, we also tested for significant differences in exposure with a chi-square test. For downstream analyses, chemical biomarkers with detectability percentages of 50% and higher were maintained.

3.2.4 Data Management and Analysis

All data management and analysis were completed in R 4.0.5 GUI 1.74 Catalina build. Graphics were created using the ggplot2 package library. All data was downloaded using the RNHANES package and included the demographic, occupational, and pesticide exposure datasets shown in Table 7. In each of the methods used for this project, the analysis was first completed in all participants by farmwork history and then completed in farmworkers only by U.S. citizenship.

First, all the demographic information was stratified by history of industry or occupational farmwork and later by U.S. citizenship. For categorical demographics like racial ethnicity or education status we completed a chi-square test to see if there were significant differences in demographics between farmworkers and non-farmworkers. For variables with low responses once stratified, we used a Fisher's exact test. For continuous variables a one way ANOVA test with two groups (equivalent to a t-test) was used, and for low response cells, a Kruskal-Wallis, which is a rank based tested for multiple-group analyses. Moreover, the data was weighted using WTMEC2YR for all data collected in 2002 or later, whereas WTMEC4YR was used for data collected between 1999 and 2002 for the demographic comparisons After completing all demographic stratified analyses in farmworkers versus non-farmworkers., we narrowed the participants to farmworkers before conducting analyses among farm workers stratified by U.S. citizenship.

3.2.5 Calculating Molarity

Using the chemical names provided in the NHANES codebook, the National Center for Biotechnology and Information's PubChem Library was searched to obtain each chemical's PubChem ID, CASRN, common name, and molecular weight. PubChem contains the largest collection of open access chemical information that is commonly found on the Material Safety Data Sheets (MSDS) as well as resources with pertinent information on the toxicity, patents, and other topics of each chemical. NHANES measurements were converted to molarity by dividing the measurement by the molecular weight of the chemical.

3.2.6 Pesticide Concentration Distribution

Once molarity was calculated using the molecular weights and CASRN's from PubChem, we created pesticide concentration distribution boxplots by the chemical and farmwork history or U.S. citizenship. These boxplots were created in the tidyverse using the *ggplot2* R package. These distributions were overlayed to the same axis and rotated 90 degrees to make it more visible to be able to directly see overlap between the pesticide concentration distributions of exposure and bioactivity.

3.2.7 Unadjusted regression models

Firstly, a linear regression was completed with each log10 transformed chemical measurement as the outcome and occupation status as the predictor. These regressions were considered the unadjusted model. For pesticides which were measured in urine the unadjusted model also controlled for urinary creatinine, and for pesticide biomarkers measured in blood, the lipid adjusted value was used [141].

3.2.8 Fully Adjusted Model

The adjusted linear regressions all control for urine creatinine or lipid adjustment, body mass index (BMI), age in years, gender, racial ethnicity, education level, poverty-income ratio (representative of socio-economic status), US citizenship, and survey year. The percent change was calculated by exponentiating 10 by the estimate and subtracting 1 from the total. To deal with the complex survey design of NHANES, we used the *survey* package in R for all analyses to account for masked variance pseudo primary sampling units (SDMVPSU), strata (SDMVSTRA), and individual sample weights.

3.3 Results

In Table 8, the demographic frequencies are presented by whether the participant had a history of farmwork or not. In total, there were 1,137 people with any farmwork history, and 20,205 who we categorized as non-farmworkers. The farmworker group was mostly men (N=697, 61.3%), Non-Hispanic White (N=635, 55.8%), U.S. Citizens (N=934, 82.1%) and were college graduates school education (N=302, 26.6%). The non-farmworker group had similar mean BMI, age, and poverty-income ratio. The non-farmworker group is predominantly men

(N=10,187, 50.4%), Non-Hispanic White (N=9,167, 45.4%), had U.S. Citizenship (N=17,626, 87.2%), and had some college or an associate degree (N=3,885, 19.2%).

In Table 9 we outline the results for detectability by chemical within NHANES. Initially, this list included 96 different chemicals present in NHANES. Farmworkers consistently had more detectable measurements of POPs when compared to non-farmworkers except for Mirex. Of the 14 chemicals above 48% detectability in Table 9, Trans-nonachlor (p=0.037), oxychlordane (p= 8.87×10^{-5}), 4-nitrophenol (p=7.5×10^{-10}), β-hexachlorocyclohexane (p= 1.48×10^{-4}), and p,p'-DDT (p=1.49×10^{-3}) were significantly higher in farmworkers than non-farmworkers. After removing only chemicals with 50% or higher detectability percentages, we were left with 14 chemicals for analysis (Table 10).

Figure 3 presents boxplots of the pesticide concentration distributions by the chemical, stratified by history of farmwork. A few chemicals' mean pesticide concentrations did differ more than others. The unadjusted model results are presented in Table 11. When only adjusting for lipids or urinary creatinine, p,p'-DDE (31.04%, p=1.7×10^{-3}), beta-hexachlorocyclohexane (24.65%, p= 6.6×10^{-3}), Oxychlordane (22.58%, p= 3.03×10^{-3}). Trans-nonachlor (14.19%, p=0.031), and 4-Nitrophenol (-29.69%, p= 1.2×10^{-5}) significantly differed between farmworkers and non-farmworkers.

In Table 12, the estimate, standard error, and p-value are presented for the fully adjusted regression model. Using the fully adjusted model that also adjusts for social determinants of health, we can see that based on the percent change the top four chemicals with the highest percent changes still significantly differed by farmwork history. Three pesticides had significantly different pesticide biomarker concentrations in farmworkers and non-farmworkers:

2,4-dichlorophenoxyacetic acid (29.72%, p=0.006), p,p'-DDT (21.1%, p=0.034), and 4-nitrophenol (-26.55%, $p=1.65 \times 10^{-4}$).

To test whether there are differences in pesticide biomarker concentrations by citizenship status, we first restricted the study population to farmworkers only. Table 13 presents the population demographic data for all farmworkers comparing US citizens and non-US citizens. Overall, 237 of the farmworkers were non- US citizens and 1,007 were US citizens. Among non-US citizens, most farmworkers identify as Mexican American (N=153, 75.4%), whereas most farmworkers who are US citizens self-identified as Non-Hispanic White (N=622, 66.6%). Additionally, US citizen farmworkers had significantly higher poverty-income ratio (mean=3.13, standard error= 1.68) than non-US citizen farmworkers (mean PIR=1.41, standard error=1.07, $p < 2.2 \times 10^{-16}$). There is also a significant difference in US citizen and non-US citizen education with most non-US citizens having less than a 9th grade education (N= 123, 60.6%) versus the majority of US citizen farmworkers having some college or an associate degree (N=290, 31.1%, $p < 2.2 \times 10^{-16}$).

Figure 4 presents boxplots of the pesticide concentrations by chemical, stratified by citizenship status. When comparing farmworkers with and without U.S. citizenship, there are some very large differences in the mean concentration for each chemical such as 2,5-Dichlorophenol where non-citizen farmworkers had a mean of 2.8uM and citizen farmworkers had a mean of 0.8uM. Additionally, p,p'-DDE and p,p'-DDT also had much higher mean concentrations in non-citizen farmworkers (150nM and 1.5nM, respectively) than citizen farmworkers (12.3nM and 1.8pM, respectively).

Table 14 presents the unadjusted regression results for each chemical, where 7 of the 14 chemicals significantly differed between individuals with and without US citizenship . These 7 significant chemicals include p,p-DDT (percent difference = 221.89%, p= 3.95×10^{-4}), p,p'-DDE (139.29%, 1.22×10^{-4}), Beta-hexachlorocyclohexane (40.79%, p=0.013), dieldrin (-29.92%, 9.73×10^{-4}), 2,4-Dichlorophenoxyacetic acid (-32.22%, 9.73×10^{-4}), oxychlordane (-51.57%, p= 6.43×10^{-7}), and trans-nonachlor (-51.61%, p=1.22×10^{-7}).

Table 15 presents the adjusted regression of each pesticide. When looking at citizenship, 2,5-Dichlorophenol (469.71%, p=2.73×10^{-8}), p,p'-DDT (150.23%, p= 3.0×10^{-4}), 2,4-Dichlorophenol (134.31%, p= 0.101) had the greatest difference in chemical concentration among farmworkers by citizenship status. In the adjusted model, non-citizens had lower exposure biomarkers in 4 chemicals, although not significantly; these chemicals include DEET acid (-41.09, p=0.150), 4-nitrophenol (-19.05%, p=0.462), trans-nonachlor (-12.06, p=0.494), and 2,4-Dichlorophenoxyacetic acid (-10.95, p=0.736).

3.4 Discussion

Pesticides are an everyday encounter for many people globally whether it is moth balls in the attic (which contain 1,4-dichlorobenzene, the parent compound for 2,5,-Dichlorophenol), spraying off our clothes before heading outdoors for a hike (DEET, parent compound for DEET acid), or spraying the edamame budding across a farm in Northern Thailand (e.g. Chlorpyrifos) [142]. However there still is limited research on how much of these chemicals U.S. residents are exposed to and how social determinants may be associated with these exposures. We proposed this study to quantify pesticide exposure among NHANES participants and to understand how these exposures may differ by history of farmwork and U.S. Citizenship. Initially, we hypothesized having a history of farmwork would be associated with a higher mean concentration when compared to non-farmworkers, and when narrowed to farmworkers only, people living in the U.S. without citizenship will average higher concentrations of pesticide exposure biomarkers.

This study started with 96 pesticide biomarkers and ended with 14 after removing chemicals with a detection frequency less than 50%. Farmworkers and Non-Farmworkers differed significantly on all demographics except for US Citizenship make up and BMI. When narrowed to farmworkers only, demographics significantly differed based on US Citizenship. People living without US citizenship were mostly less than 9th grade educated (N=271, 66.7%), predominantly Mexican American (N=378, 81.1%), had significantly lower poverty-income ratios (1.3 vs 3.03), and were significantly smaller in BMI (27 versus 28.7 kg/m^2). Overall, our hypothesis was supported for specific chemicals. For example, worker category was found to significantly predict p,p'-DDT, 2,4 Dichlorphenoxyacetic acid, and 4-nitrophenol. Moreover, citizenship appeared to present significant differences among farmworker pesticide exposure with 2,5-Dichlorophenol and p,p-DDT.

3.4.1 Immigrant Health

Much of the research on farmworkers working in the US without citizenship focuses on migrant workers and can help to inform some differences in exposure. Among migrant workers, there tends to be limited access to healthcare, lower education, and lower pay which creates unique vulnerabilities for farmworkers without citizenship and who travel for contract farmwork[17,140,143]. This directly reflects the statistically significant differences our study found. Previous research has found farmworkers living without citizenship have worsened health outcomes and prognoses [76,79,80]. In our study, we found that citizenship among farmworkers resulted in differential pesticide exposure concentration distributions which may, in part, be driving disease outcome disparities.

A study using NHANES data from 2011 to 2016 retrieved 16,986 adults age 20 years and older[79]. This study stratified participants into the following three citizenship categories: USA-born citizen, Foreign-born citizens, and non-citizens[79]. These stratified groups were then analyzed for diabetes, hypertension, or hypercholesterolemia prevalence, treatment, and control[79]. While this study did not investigate occupation or history of farmwork, they did find statistically significant differences in health outcomes by citizenship. In particular, immigrants regardless of citizenship have higher prevalence of diabetes than U.S. Born Citizens (15.7% versus 12.8%, $p<0.001$)[79]. For people residing in the U.S. without citizenship, [79]. Furthermore, immigrants living in the U.S. without citizenship had statistically significant (p-value < 0.001) lower treatment rates of hypercholestermia (16.4% vs. 45.5% and 43.3%), hypertension (60.3% vs. 81.1% and 79.6%), and diabetes (51.2% vs. 69.5% and 66.6%) than US-Born and Immigrants living with Citizenship[79]. Studies have also found that other factors directly like food insecurity, disability, or health insurance status are also associated with residing in the US without US citizenship[76,80].

Researchers have looked at nativity, food insecurity, and time in the U.S. to understand if disability operates as a predictor among respondents between 15 and 59 years of age[80]. Altman et al. (2020) further divide respondents into four groups: 1) U.S. Born citizens, 2) Non-US Born Citizens (5 or more years in the U.S.), and people residing in the U.S. without U.S. Citizenship for 3) five or less years and 4) more than five years. U.S.-born citizens reported food insecurity the least (13.7%) and were followed by immigrant U.S. Citizens 14.9%[80]. People residing in the U.S. without U.S. citizenship reported food insecurity the most[80]. This significant difference by citizenship status increases with time in the U.S. (20.6% for less than 5 years versus 29.1% for 5 or more years) [80].

3.4.2 Farmworker Health

The 2015-2016 US Department of Labor's National Agriculture Workers Study (NAWS) recruited 5,342 crop farmworkers [144]. These farmworkers were predominantly Hispanic (83%) [144]. Most farmworkers were born in Mexico (69%) and 35% of all farmworkers born in the US were Hispanic [144]. They had a median age of 38 years, mostly identified with the open ended "other" race category (73%) while 6% of farmworkers also identified as indigenous [144]. A little over half of the farmworkers had work authorization (51%), 71% of all farmworkers were not US citizens [144]. It is also important to understand that 21% of all farmworkers were legal permanent residents and on average had been in the US for 18 years, 78% of farmworkers born outside of the US had been in the US for at least 10 years [144]. This somewhat differs from NHANES since our study was predominantly Non-Hispanic White, U.S. Citizens, and born within the US [144].

Farmworkers face unique challenges to health often due to housing and lack of access to healthcare[85,145]. A study using the United Farm Workers (UFW) of America data of 6.2 million farmworkers in the U.S. between 1973 and 2000, found 3,977 farmworkers dying from all causes[146]. When comparing the death rates of Hispanic farmworkers to the California Hispanic Population, mortality due to tuberculosis (Proportionate Mortality Rate (PMR)=2.62, 95% CI: 1.31, 4.69), cerebrovascular disease (PMR=1.53, 95%CI 1.32-1.77), and unintentional deaths (PMR=1.47, 95%CI: 1.29,1.66) were elevated in farmworkers [146]. Next, researchers compared UFW mortality to U.S. deaths and found that farmworkers had significantly higher PMRs for respiratory tuberculosis, malignant neoplasms of the stomach, biliary passes , uterine cervix, and liver and gallbladder[146]. Additionally, mortality due to diabetes mellitus , cerebrovascular disease, cirrhosis of the livers and other diseases of digestion were elevated among farmworkers in comparison to the US population. And finally, death due to unintentional poisoning, machine injuries and assault and homicide were also elevated among farmworkers, even after adjusting for racial ethnicity and sex[146].

3.4.3 Chronic Illness and Pesticide Exposure

Research on farmworkers, pesticide exposures, and cancer is difficult due to the transitory life that many farmworkers lead which can decrease the number of participants recruited and increase loss to follow up[147]. However, in a comprehensive review of cancer studies on pesticide exposure, heptachlor, lindane, 24D, chlordane, and multiple other pesticides have been associated with cancer [147]. More specifically, farmworkers recruited from membership pool of the United Farm Workers of America (UFW) were exposed to heptachlor were 2.01 times more likely to be diagnosed with prostate cancer than people not exposed to heptachlor (95% CI: 1.12-3.60, p=0.003) [147]. Among UFW members, exposure to both 24DCP and chlordane are positively associated with breast cancer (OR=2.14, OR=3.85, respectively) and stomach cancers (OR=1.85, OR=2.96, respectively)[147]. Also, farmworkers exposed to 24DCP were 3.8 times more likely to have Non-Hodgkin's lymphoma than farmworkers not exposed to 2,4-D[147].

We found an association between dichlorophenol biomarker concentrations for people and farmworkers living without US citizenship. A study on urinary dichlorophenol pesticides and obesity among adults used NHANES laboratory data from 2005-2008 and found obese adults had significantly increased concentrations of 25DCPCP ($p<0.0001$) and 24DCP ($p=0.0170$) in comparison to non-obese participants[126]. When these researchers further stratified the urinary concentration distribution by the quartile, a dose-dependent effect was found between obesity and 25DCPCP biomarker concentrations[126]. To clarify, urinary concentrations of 25DCP were found to have a significant positive association with obesity in the second interquartile for the fully adjusted odds ratios (AOR) (AOR: 1.47, 95% CI: 1.12, 1.93), third (AOR: 1.41, 95% CI: 1.07, 1.87), and fourth (AOR: 1.62, 95% CI: 1.21, 2.17) biomarker concentration quartiles after adjusting for demographics, total fat intake, and physical activity[126]. This differs from the results presented in our study. However, our study did not support this link when we adjusted for BMI and found non-citizen farmworkers had significantly lower BMIs in comparison to citizen farmworkers. We did not stratify pesticide concentration distributions by BMI across farmwork history categories.

3.4.4 Persistent Organic Pesticides

Persistent organic pesticides are chemicals with a very long half-life and can therefore last in the human body or the environment for years or longer. In a study of 122 persistent environmental pollutants including organochlorines, 156 people living with ALS and 128 people living without ALS were recruited [70]. Amyotrophic lateral sclerosis (ALS) is a disease characterized by selective degeneration of motor neurons and thus far the etiological drivers for the disease are still not fully understood. Any pesticide exposure was significantly associated with ALS (OR=5.09, P=0.002) [70]. More specifically, when using a multivariable model to represent cumulative exposure, cis-chlordane was significantly associated ALS (OR=6.51, p-value=0.002) [70].

Researchers investigating organochlorine exposure and dementia retrieved information on 669 clinically assessed participants using data from the Canadian Study of Health and Aging (CSHA)[127]. In this study researchers used the Modified Mini-Mental State Examination to determine global cognitive function among individuals age 65 and older over 10 years[127]. In this study, researchers created two models: Model 1 which was adjusted for total lipids and CSHA phase, age, sex, education, and apolipoprotein E allele e4; and Model 2 which was further adjusted for BMI, smoking, alcohol drinking, residence area, vascular score, copper, lead, mercury, and zinc[127]. The beta coefficients represent individual OC compound concentrations in relation to 3MS scores[127]. Based on Model 2, Medehouenou et al. (2019) found that among people who developed Alzheimer's Disease over the course of the study (N=136) who were exposed to p,p'-DDT and p,p'-DDE had significantly declined cognitive function based on the 3MS scores (β=-1.7, $p<0.0001$ and β=-1.6, $p<0.0001$, respectively) [127]. In our study, farmworkers had significantly higher biomarker concentrations of p,p-DDE, oxychlordane, and DEET acid. Furthermore quantifying pesticide exposure in NHANES by US citizenship status also showed significant elevation of biomarkers of beta-hexachlorocyclohexane, p,p-DDT, p,p-DDE, trans-nonachlor, and other persistent pesticides in non-US citizen farmworkers.

In a study of persistent organic pollutants and mortality in NHANES from 1999 to 2011, researchers retained respondents who were 60 years of age and older and had measurements of four organochlorine pesticide biomarkers, with at least 90% detectability for all participants (N=1,428 participants)[148]. Beta-hexachlorocyclohexane biomarker concentrations were associated with increased risk of all-cause mortality among older Americans [HR per 1 SD increase=1.18, 95% CI = 1.01, 1.38] [148]. Additionally, other cause mortality risk had a positive association with oxychlordane [HR = 1.15 95% CI 1.06, 1.25], p,p'-DDE [HR = 1.12, 95% CI =

1.02, 1.23], trans-nonachlor [HR = 1.11, 95% CI = 1.04, 1.18], and beta-hexachlorocyclohexane [HR = 1.25, 95% CI = 1.03, 1.52] [148]. This same study also found that participants exposed to four organochlorine pesticides or more were also at a higher risk for non-cancer, non-heart/cerebrovascular disease mortality[148].

Furthermore, a study analyzing data from the NHANES 2003-2004 cycles found higher white blood cell counts were seen in people with higher biomarker concentrations trans-nonachlor and oxychlordane[149]. Additionally, this relationship was found to be dose-responsive, in that an increased trans-nonachlor concentration was associated with increased lymphocytes and neutrophils counts [149]. In this same study, oxychlordane was significantly positively associated with lymphocyte, segmented neutrophils and white blood cell counts[149]. This data suggests that trans-nonachlor and oxychlordane also have effects on the humans [149].

3.4.5 Limitations and Strengths

Our research shows that NHANES respondents are exposed to multiple pesticides and pesticide types. Quantifying chemical mixtures across a population is complex and methodology for understanding these mixtures is still an emerging area of research. However, there is still plenty of research to be done in understanding chemical mixtures. Much of the research on chemical health outcomes focuses on one chemical at a time, including our study, but people are often exposed to more than one chemical, chemicals can interact with each other to create new chemicals and once chemicals are in the environment, they can also react with the ambient air or be degraded by the sun’s rays. All the changes to chemicals in relation to mixtures and being in the environment create nuanced exposures and further research is needed to understand how these mixtures may uniquely affect the human body.

First and foremost, it is important to understand there is a possibility for misclassification between the work categories. Since NHANES is not intended for agriculture health research, the occupation and industry title used to categorize respondents as farmworker or no farmwork was “Agriculture, Forestry, and Fishing”. This means that some people without farmwork history may be included in the ‘farmworkers’ and people in the ‘non-farmworkers’ group may also work in an industry that involves pesticide spraying or exposure (*e.g.* pesticide manufacturing and some military personnel). This type of misclassification will likely bias the results towards the null.

Additionally, oxypyrimidine (7.88% vs. 13.76%, 0.033), desethyl hydroxy DEET (17.37% vs. 11.30%, p =0.015), and DEET (9.17% vs 6.25%, p = 0.036) had significantly different frequencies of detection between farmworkers and non-farmworkers, respectively. These chemicals had detectability percentages below the cutoff for inclusion in our study, and thus were not analyzed further. However, it is possible that by restricting the chemicals included by detectability percentage, we are possibly missing some important differences in pesticide exposure between farmworkers and non-farmworkers. Identifying individuals who may be at particularly high risk of exposures to these chemicals would be an important future direction of research.

One of the major limitations of this project is the data on NHANES is only full through 2014 at the time analysis was completed in August 2020. While NHANES is thorough, reliable, and valid study, it is still a cross-sectional study. This means the measurements within it are a single measurement in time and cannot be fully representative of chronic exposures or chronic symptomology due to exposures. Another limitation includes the majority of farmworkers being recruited between 1999 and 2004 (N= 1,775, 69.6%), which is of importance since the recruitment and laboratory methods have been updated since 2003. Newer methods for quantifying chemicals from blood and urine samples are more sensitive and can detect lower quantities of chemicals. Additionally, farmworkers living without citizenship had significantly lower BMI as well, which can cause changes in metabolism and concentration of chemicals stored in the body.

Our study is the first study to provide a comprehensive quantification of all the pesticide exposure concentrations within the US population using NHANES from 1999 to 2014 and to then stratify these concentrations by social determinants of health with a focus on farmwork, fishing, and forestry work history and U.S. citizenship. By considering all the pesticides within NHANES and narrowing down to those with at least 50% detectability, we find that even within NHANES a small portion (15%) of chemicals are detected in a majority of NHANES participants. Additionally, this study is one of few to consider health disparities associated with occupation or citizenship and how they may affect pesticide exposure.

3.4.6 Future Directions

More research on the chemicals this study found to be disparate between farmworkers with and without citizenship needs to be completed to ensure all farmworkers in the US are protected from the health harms of concentrated pesticide exposure. This study provides pesticide exposure concentration distributions in the US population, and the most logical next step is to further understand whether these chemical concentrations are high enough to induce bioactivity for each chemical. Furthermore, by stratifying exposure and bioactivity concentrations by social determinants of health—like occupation and citizenship—will further assist the field in understanding how disparities in health outcomes may be mechanistically linked to occupational exposures, like pesticides. To improve the lives of disparate people and all people living within the US, we must address the systemic barriers to protective laws and access to preventive measures.

3.5 Figures

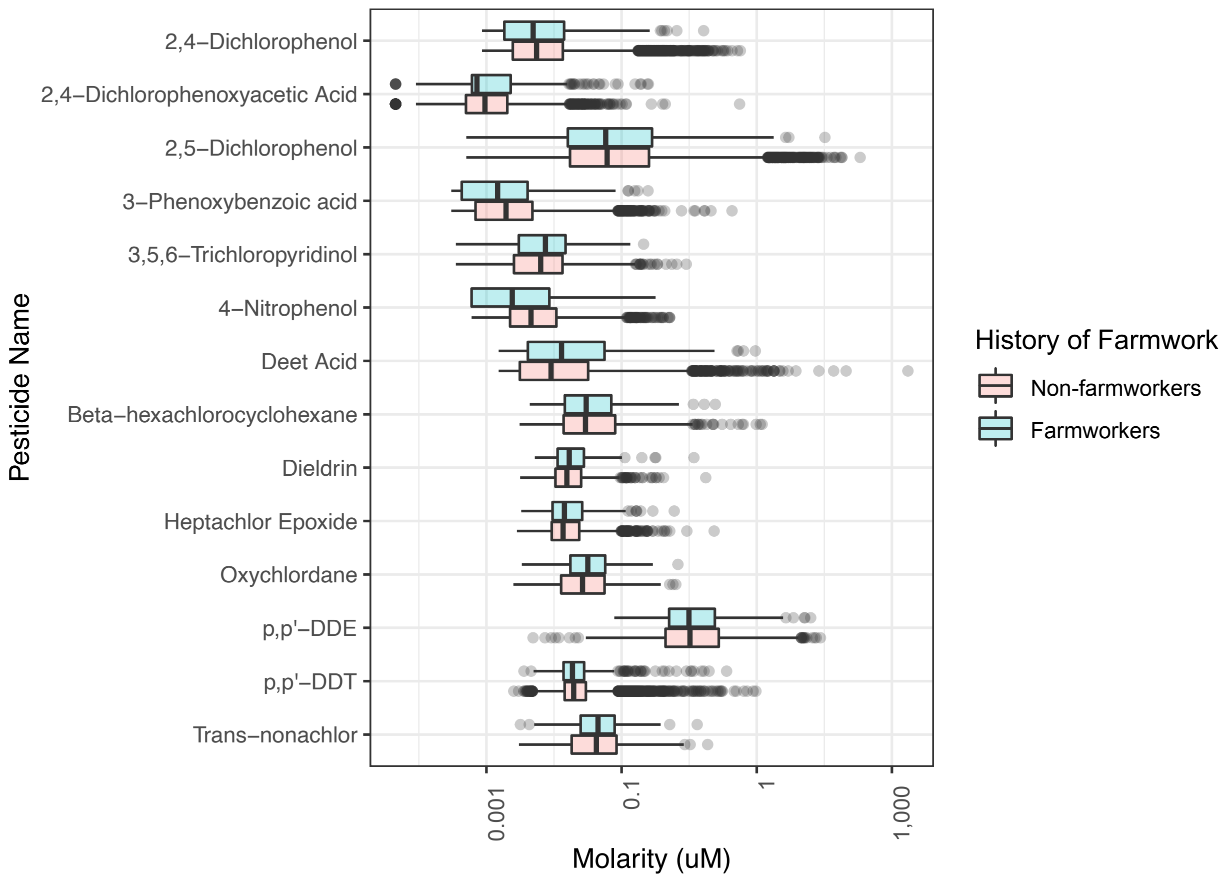


Figure 3 1999-2014 Pesticide Concentration Distribution in NHANES, stratified by history of farmwork

From the Industry and Occupation Survey, individuals were coded as "farmworker" or "non-farmworker" using the Current Industry (OCD231=1), Current Occupation (OCD241=18), Longest Industry (OCD391=1), and Longest Occupation (OCD392=18), where all participants who put "Agriculture, Forestry and Fishing" was coded as a farmworker. NHANES measurements were converted to molarity by dividing the measurement by the molecular weight of the chemical.

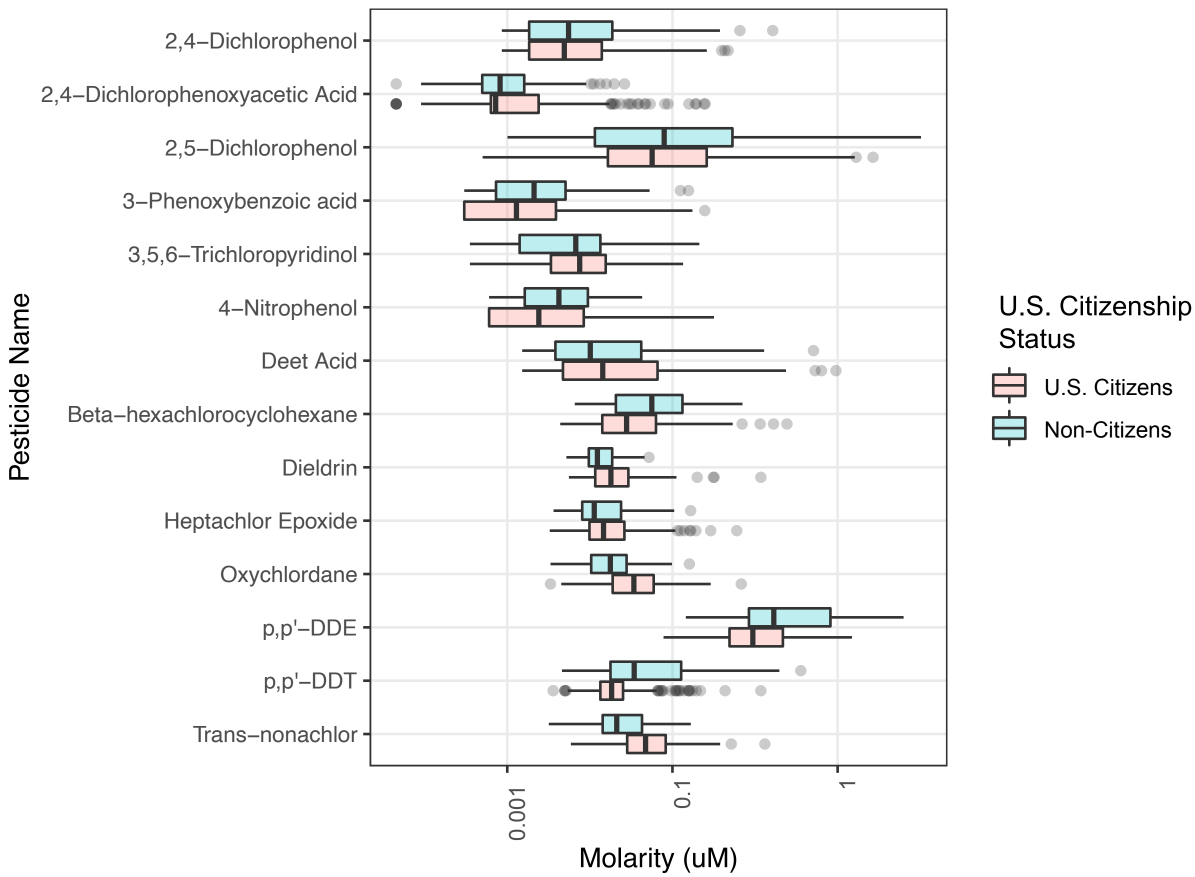


Figure 4 1999-2014 Pesticide Concentration Distribution in NHANES among Farmworkers, stratified by U.S. Citizenship Status

From the Industry and Occupation Survey, individuals were coded as “farmworker” or “non-farmworker” using the Current Industry (OCD231=1), Current Occupation (OCD241=18), Longest Industry (OCD391=1), and Longest Occupation (OCD392=18), where all participants who put “Agriculture, Forestry and Fishing” was coded as a farmworker. NHANES measurements were converted to molarity by dividing the measurement by the molecular weight of the chemical.

3.6 Tables

Table 7 NHANES Datasets included in this study

Dataset Name	*Dataset File Name*	*Years*
Organophosphates	OPD_D	2005-2006
	OPD_E	2007-2008
	OPD_G	2011-2012
Carbamates	CARB_D	2005-2006
	CARB_E	2007-2008
Persistent Pesticides	LAB26PP	1999-2000
	L26PP_B`	2001-2002
	L26UPP_C	2003-2004
	UPP_D	2005-2006
	UPP_E	2007-2008
Priority Pesticides (Household)	L24PP_C	2003-2004
	PP_D	2005-2006
	PP_E	2007-2008
	PP_F	2009-2010
	PP_G	2011-2012
	LAB28POC	1999-2000
Persistent Organochlorines	L28POC_B	2001-2002
	L28OCP_C	2003-2004
Non-persistent pesticides	UPHOPM_F	2009-2010
	UPHOPM_G	2011-2012
	UPHOPM_H	2013-2014
DEET and DEET Metabolites	DEET_E	2007-2008
	DEET_F	2009-2010
	DEET_G	2011-2012
	DEET_H	2013-2014
Atrazine and Atrazine Metabolites	UAM_E	2007-2008
Occupation	OCQ	1999-2000
	OCQ_B	2001-2002
	OCQ_C	2003-2004
	OCQ_D	2005-2006
	OCQ_E	2007-2008
	OCQ_F	2009-2010
	OCQ_G	2011-2012
	OCQ_H	2013-2014
Demographics	DEMO	1999-2000

	DEMO_B	2001-2002
	DEMO_C	2003-2004
	DEMO_D	2005-2006
	DEMO_E	2007-2008
	DEMO_F	2009-2010
	DEMO_G	2011-2012
	DEMO_H	2013-2014
Body Mass Index	BMX	1999-2000
	BMX_B	2001-2002
	BMX_C	2003-2004
	BMX_D	2005-2006
	BMX_E	2007-2008
	BMX_F	2009-2010
	BMX_G	2011-2012
	BMX_H	2013-2014

Table 8 Stratified Demographics of NHANES Participants, by Farmwork Category

	Non-Farmworker		*Farmworker*		
Variable	*Mean*	*Standard Error*	*Mean*	*Standard Error*	*p-value*
Body Mass Index	28.4	6.7	28.32	6.08)	0.584
Age in years	45.88	19.5	48.63	18.81	2.15x10-4
Poverty-Income Ratio	2.5	1.63	2.82	1.72)	5.99x10-5
Survey Year	*N=20,205*	*Percent*	*N=1,137*	*Percent*	*< 2.2x10-16*
1999-2000	1,404	6.9	159	14	
2001-2002	1,691	8.4	219	19.3	
2003-2004	2,890	14.3	358	31.5	
2005-2006	1,654	8.2	32	2.8	
2007-2008	3,626	17.9	87	7.7	
2009-2010	3,831	19	154	13.5	

2011-2012	3,278	16.2	96	8.4	
2013-2014	1,831	9.1	32	2.8	
Gender					1.76x10-10
Men	10,187	50.4	440	38.7	
Women	10,018	49.6	697	61.3	
Racial Ethnicity					< 2.2x10-16
Mexican American	3,517	17.4	278	24.5	
Other Hispanic	1,577	7.8	36	3.2	
Non-Hispanic White	9,167	45.4	635	55.8	
Non-Hispanic Black	4,435	22	135	11.9	
Other Race	1,509	7.5	53	4.7	
Country of Birth					0.538
Born in 50 US states or DC	606	90.2	0	-	
Born in Mexico	30	4.5	71	74	
Born elsewhere	36	5.4	25	26	
U.S. Citizenship					4.04x10-4
Non-Citizen	2579	12.8	203	17.9	
Citizen	17626	87.2	934	82.1	
Education Level					< 2.2x10-16
Less than 9th grade	2004	9.9	233	20.5	
9-11th grade	4021	19.9	147	13	
Highschool	4707	23.3	210	18.5	
Graduate/GED	5566	27.6	243	21.4	
Some College or AA	3885	19.2	302	26.6	

P-values are derived from a chi-square test, using a Yate's Correction where necessary, and for continuous variables, a Wilcoxon Rank Test was used with a Kruskall-Wallis Correction (as needed).

Percentages are out of the total number of respondents for that specific question.

In this table, other race includes multi-racial.

In this study, 9-11 grad includes 12th grade completion without a high school diploma.

All values in this dataset are weighted and stratified according to NHANES guidelines.

Table 9 Detectability of Chemicals by Work Category

Comment Code	Chemical Name	Non-Farmworkers			Farmworkers			p-value
		Below LOD	Above LOD	% Above LOD	Below LOD	Above LOD	% Above LOD	
LBXPDE	p,p'-DDE	7	4,455	99.84	-	274	100.00	1
URX14D	2,5-Dichlorophenol	157	8,513	98.19	7	278	97.54	0.369
URXDCB	2,4-Dichlorophenol	1,076	7,594	87.59	42	243	85.26	0.237
LBXTNA	Trans-nonachlor	609	3,828	86.27	25	245	90.74	0.035
URXCPF	Chlorpyrifos	903	5,536	85.98	47	216	82.13	0.086
URXCPM	3,5,6-Trichloropyridinol	903	5,536	85.98	47	216	82.13	0.086
URXCPO	Chlorpyrifos-oxon	903	5,536	85.98	47	216	82.13	0.086
URXDEA	Deet Acid	1,327	5,811	81.41	28	164	85.42	0.187
URXOPM	3-Phenoxybenzoic acid	1,537	6,501	80.88	71	243	77.39	0.126

LBXOXY	Oxychlordane	1,036	3,134	75.16	37	219	85.55	$8.87x10^{-5}$
URXPAR	4-Nitrophenol	2,049	5,862	74.10	130	175	57.38	$7.5 x10^{-10}$
LBXDIE	Dieldrin	849	2,144	71.63	51	144	73.85	0.566
LBXBHC	Beta-hexachlorocyclohexane	1,350	3,067	69.44	53	215	80.22	$1.48 x10^{-4}$
URX24DCP	2,4-Dichlorophenoxyacetic acid	3,713	5,825	61.07	165	227	57.91	0.224
LBXHPE	Heptachlor Epoxide	1,950	2,171	52.68	107	153	58.85	0.055
LBXPDT	p,p'-DDT	2,164	2,047	48.61	109	155	58.71	$1.49 x10^{-3}$
		Below 48% Detectable						
URXCCC	Cis Dichlorovnl-Dimeth Carboacid	1,667	1,040	38.42	101	64	38.79	0.934
LBXHCB	Hexachlorobenzene	2,703	1,572	36.77	167	99	37.22	0.896
URXALA	Alachlor mercapture	613	334	35.27	31	17	35.42	1
URXPCP	Pentachlorophenol	1,016	486	32.36	51	29	36.25	0.465
URX1TB	2,4,5-Trichlorophenol	4,781	2,221	31.72	156	84	35.00	0.291
LBXMIR	Mirex	3,107	1,248	28.66	196	73	27.14	0.627

URX3TB	2,4,6-Trichlorophenol	5,003	1,999	28.55	177	63	26.25	0.468
URXMAL	Malathion Diacid	2,079	783	27.36	82	33	28.70	0.750
URXOPP	O-Phenyl Phenol	5,227	1,775	25.35	168	72	30.00	0.114
URXOXY	Oxypyrimidine	4,226	1,121	20.97	121	26	17.69	0.410
URXTCC	Desisopropyl Atrazine	6,348	1,593	20.06	240	70	22.58	0.279
URXDIZ	Oxypyrimidine	2,268	362	13.76	152	13	7.88	0.033
URXDHD	Desethyl Hydroxy Deet	6,345	808	11.30	157	33	17.37	0.015
URXETU	Ethylenethio urea	4,453	428	8.77	161	12	6.94	0.492
URX4FP	Fluoro-Phenoxybenzoic acid	7,478	591	7.32	296	16	5.13	0.180
URXDEE	DEET	9,264	618	6.25	327	33	9.17	0.036
URXDPY	Diethylaminomethylpyrimidinol/ One	1,616	106	6.16	109	6	5.22	0.841
URXMET	Metolachlor Mercapturate	1,690	65	3.70	116	4	3.33	1
URXCMH	Chloro-Hydro-Meth-Chromen-One/Ol	1,647	62	3.63	114	3	2.56	0.796
LBXODT	o,p'-DDT	4,029	139	3.33	251	11	4.20	0.478

URXCBF	Carbofuranphenol	4,109	129	3.04	237	9	3.66	0.567
URX25T	2,4,5-Trichlorophenoxyacetic acid	5,926	165	2.71	310	6	1.90	0.476
URXACE	Acetochlor Mercapturate	1,686	43	2.49	118	1	0.84	0.361
URXAPE	Acephate	4,919	92	1.84	179	4	2.19	0.582
LBXGHC	Gamma-hexachlorocyclohexane	4,240	70	1.62	258	8	3.01	0.135
URXATZ	Atrazine mercapture	4,399	50	1.12	206	4	1.90	0.307
URXCB3	Deisopropyl Atrazine Mercapture	4,474	36	0.80	223	2	0.89	0.702
URXDCZ	Diaminochloroatrazine	1,821	7	0.38	45	1	2.17	0.181
URXPPX	2-Isopropoxyphenol	4,163	16	0.38	237	4	1.66	0.021
URXMMI	Methamidaphos	5,004	19	0.38	176	1	0.56	0.500
URXMTO	Dimethoate	5,080	13	0.26	182	-	0.00	1
URXPTU	Propylenethio urea	5,086	9	0.18	185	-	0.00	1
LBXALD	Aldrin	3,063	5	0.16	196	1	0.51	0.312
URXEMM	Ethametsulfuron Methyl	4,902	8	0.16	176	-	0.00	1

URXNOS	Nicosulfuron	4,845	7	0.14	176	-	0.00	1
URXDTZ	Desethyl Atrazine	1,753	2	0.11	44	1	2.22	0.073
LBXEND	Endrin	2,913	3	0.10	191	1	0.52	0.225
URXSIS	Desisopropyl Atrazine	1,722	1	0.06	44	-	0.00	1
URXSIM	Desisopropyl Atrazine Mercapturate	1,752	1	0.06	45	1	2.17	0.051
URXOMO	O-methoate	5,097	2	0.04	182	-	0.00	1
URXCHS	Chloro Sulfuron	4,725	1	0.02	169	-	0.00	1
URXMTM	Metsulfuron Methyl	4,964	1	0.02	180	-	0.00	1
URXSSF	Sulfosulfuron	4,999	1	0.02	181	-	0.00	1
URXOXS	Oxasulfuron	5,013	1	0.02	181	-	0.00	1
URXAAZ	Atrazine	1,828	-	0.00	46	-	0.00	1
URXBSM	Bensulfuron Methyl	4,974	-	0.00	181	-	0.00	1
URXFRM	Foramsulfuron	4,780	-	0.00	174	-	0.00	1
URXHLS	Halosulfuron	4,903	-	0.00	181	-	0.00	1

URXMSM	Mesosulfuron Methyl	5,020	-	0.00	181	-	0.00	1
URXPIM	Primisulfuron Methyl	4,735	-	0.00	174	-	0.00	1
URXPRO	Prosulfuron	4,843	-	0.00	176	-	0.00	1
URXRIM	Rimsulfuron	4,938	-	0.00	175	-	0.00	1
URXSMM	Sulfometuron Methyl	4,759	-	0.00	169	-	0.00	1
URXTHF	Thifensulfuron Methyl	4,974	-	0.00	179	-	0.00	1
URXTRA	Triasulfuron	4,872	-	0.00	178	-	0.00	1
URXTRN	Triflusulfuron Methyl	4,967	-	0.00	183	-	0.00	1

NHANES is an abbreviation for the National Health and Nutrition Examination Survey, a cross-sectional study of people residing in the United States and maintained by the Centers for Disease Control and Prevention. The above chemicals are included based on respondent (unique SEQN) who also had occupation data and laboratory results data collected.

Table 10 Pesticides with more than 50% detectability in NHANES, 1999-2014

Variable Name	*Chemical Name*	*Units in NHANES*	*CASRN*
URXDCB	2,4-Dichlorophenol	µg/L	120-83-2
URX24DCP	2,4-Dichlorophenoxyacetic Acid	µg/L	94-75-7
URX14D	2,5-Dichlorophenol	µg/L	583-78-8
URXCPM	3,5,6-Trichloropyridinol	µg/L	6515-38-4
URXPAR	4-Nitrophenol	µg/L	100-02-7
URXDEA	DEET Acid	µg/L	134-62-3
URXOPM	3-Phenoxybenzoic acid	µg/L	3739-38-6
URXCPF	Chlorpyrifos*	µg/L	2921-88-2
URXCPO	Chlorpyrifos-oxon*	µg/L	5598-15-2
LBXBHC	β-hexachlorocyclohexane	ng/g	319-85-7
LBXDIE	Dieldrin	ng/g	60-57-1
LBXHPE	Heptachlor Epoxide	ng/g	76-44-8
LBXTNA	Trans-nonachlor	ng/g	39765-80-5
LBXPDE	p,p'-DDE	ng/g	72-55-9
LBXPDT	p,p'-DDT	ng/g	50-29-3

NHANES is an abbreviation for the National Health and Nutrition Examination Survey, a cross-sectional study of people residing in the United States and maintained by the Centers for Disease Control and Prevention. The above chemicals are included based on respondent (unique SEQN) who also had occupation data and laboratory results data collected

Table 11 Unadjusted linear regression coefficients of farmwork category variable when predicting chemical concentration

Common Name	*Units*	*Percent Change*	*Beta Estimate*	*Standard Error*	*p-value*	
Deet Acid	ng/g	58.17	0.20	0.10	0.060	
p,p'-DDE	ng/g	31.04	0.12	0.04	1.7×10^{-3}	**
ß-hexachlorocyclohexane	ng/g	24.65	0.10	0.03	6.6×10^{-3}	**
Oxychlordane	ng/g	22.58	0.09	0.03	3.03×10^{-3}	**
2,4-Dichlorophenoxyacetic acid	µg/L	19.20	0.08	0.05	0.095	
p,p'-DDT	ng/g	18.51	0.07	0.04	0.067	
Trans-nonachlor	ng/g	14.19	0.06	0.03	0.031	*
Heptachlor Epoxide	ng/g	11.91	0.05	0.03	0.071	
2,4-Dichlorophenol	µg/L	9.16	0.04	0.05	0.407	
2,5-Dichlorophenol	µg/L	8.81	0.04	0.07	0.593	
Dieldrin	ng/g	7.99	0.03	0.02	0.156	
3,5,6-Trichloropyridinol	µg/L	3.38	0.01	0.03	0.581	
3-Phenoxybenzoic acid	ng/g	-13.08	-0.06	0.04	0.097	
4-Nitrophenol	ng/g	-29.69	-0.15	0.03	1.2×10^{-5}	***

Significance codes: '***' 0.001 '**' 0.01 '*' 0.05

These models are comparing farmworkers and non-farmworker pesticide exposure by the pesticide of interest. Each model in this table is as follows: chemical concentration =intercept+ β worker category+ β_2 urinary creatinine (used for non-persistent chemicals). Using this model, β estimate and β standard error are presented in the above table. Farmwork is defined as 1=history of farmwork and 0=no history of farmwork.

The coefficient estimates and standard error for farmworker category are presented in the above table.

Since $\log_{10}$ transformation of the chemical concentration was completed prior to creating the model, percent change was created by exponentiating 10 to the chemical Beta estimate, subtracting 1 and multiplying by 100.

Table 12 Adjusted linear regression coefficients of farmwork category variable when predicting chemical concentration

Common Name	*Units*	*Percent Difference*	*Estimate*	*Standard Error*	*p-value*	
DEET Acid	ng/g	55.00	0.19	0.10	0.059	
2,4-Dichlorophenoxyacetic acid	µg/L	29.72	0.11	0.04	0.006	**
p,p'-DDT	ng/g	21.10	0.08	0.04	0.034	*
p,p'-DDE	ng/g	14.77	0.06	0.03	0.068	
2,5-Dichlorophenol	µg/L	8.25	0.03	0.06	0.559	
Beta-hexachlorocyclohexane	ng/g	7.56	0.03	0.02	0.197	
2,4-Dichlorophenol	µg/L	6.56	0.03	0.04	0.538	
Oxychlordane	ng/g	3.96	0.02	0.02	0.380	
3-Phenoxybenzoic acid	µg/L	2.72	0.01	0.04	0.742	
Heptachlor Epoxide	ng/g	1.89	0.01	0.02	0.714	
Dieldrin	ng/g	1.04	4.5x10-3	0.02	0.817	
Trans-nonachlor	ng/g	-2.92	-0.01	0.02	0.513	
3,5,6-Trichloropyridinol	µg/L	-9.81	-0.04	0.03	0.111	
4-Nitrophenol	µg/L	-26.55	-0.13	0.03	1.65×10^{-4}	***

Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05

These models are comparing farmworkers and non-farmworker pesticide exposure by the pesticide of interest. This model is adjusted for citizenship status, body mass index, age, gender, racial ethnicity, survey year, creatinine or lipid, federal poverty-income ratio, and education level.

Farmwork is defined as 1=history of farmwork and 0=no history of farmwork.

The coefficient estimates and standard error for farmworker category are presented in the above table.

Since $\log_{10}$ transformation of the chemical concentration was completed prior to creating the model, percent change was created by exponentiating 10 to the chemical Beta estimate, subtracting 1 and multiplying by 100

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Table 13 Stratified Demographics of NHANES Participants with a History of Farmwork, by Citizenship

		Citizen		*Non-Citizen*		
Variable		*Mean*	*Standard Error*	*Mean*	*Standard Error*	*p-value*
Body Mass Index		28.52	6.33	27.37	4.66	0.038
Age in years		49.9	18.9	42.74	17.07	7.57×10^{-5}
Poverty- Income Ratio		3.13	1.68	1.41	1.07	$< 2.2 \times 10^{-16}$
Variable		N=1,007	%	N=237	%	
Survey Year	*1999-2000*	139	14.9	20	9.9	9.41×10^{-05}
	2001-2002	188	20.1	31	15.3	
	2003-2004	325	34.8	33	16.3	
	2005-2006	20	2.1	12	5.9	

	2007-2008	66	7.1	21	10.3	
	2009-2010	105	11.2	49	24.1	
	2011-2012	68	7.3	28	13.8	
	2013-2014	23	2.5	9	4.4	
Gender	*Men*	378	40.5	62	30.5	0.013
	Women	556	59.5	141	69.5	
Racial Ethnicity	*Mexican American*	125	13.4	153	75.4	$< 2.2 \times 10^{-16}$
	Other Hispanic	23	2.5	13	6.4	
	Non-Hispanic White	622	66.6	13	6.4	
	Non-Hispanic Black	129	13.8	6	3	
	Other Race	35	3.7	18	8.9	
Country of Birth	*Born in 50 US states or DC*	606	90.2	0	0	$< 2.2 \times 10^{-16}$
	Born in Mexico	30	4.5	71	74	
	Born elsewhere	36	5.4	25	26	
Education Level	*Less than 9th grade*	110	11.8	123	60.6	$< 2.2 \times 10^{-16}$
	9-11th grade	115	12.3	32	15.8	
	Highschool	183	19.6	27	13.3	
	Graduate/GED	234	25.1	9	4.4	
	Some College or AA	290	31.1	12	5.9	

P-values are derived from a chi-square test, using a Yate's Correction where necessary, and a Wilcoxon Rank Test was completed with a Kruskall-Wallis Correction. Percentages are out of the total number of respondents for that specific question. In this table, other race includes multi-racial. In this study, 9-11 grad includes 12th grade completion without a high school diploma.

Table 14 Unadjusted linear regression coefficients of citizenship variable when predicting chemical concentration

Common Name	*Units*	*Percent Change*	*Beta Estimate*	*Standard Error*	*p-value*	
p,p'-DDT	ng/g	221.89	0.51	0.13	3.95×10^{-4}	***
p,p'-DDE	ng/g	139.29	0.38	0.11	1.22×10^{-4}	**
2,5-Dichlorophenol	µg/L	70.85	0.23	0.16	0.159	
2,4-Dichlorophenol	µg/L	47.25	0.17	0.10	0.109	
Beta-hexachlorocyclohexane	ng/g	40.79	0.15	0.06	0.013	*
4-Nitrophenol	µg/L	15.19	0.06	0.06	0.311	
3-Phenoxybenzoic acid	µg/L	9.77	0.04	0.07	0.550	
Heptachlor Epoxide	ng/g	-18.48	-0.09	0.06	0.156	
3,5,6-Trichloropyridinol	µg/L	-26.27	-0.13	0.08	0.096	
Dieldrin	ng/g	-29.92	-0.15	0.04	9.73×10^{-4}	***
2,4-Dichlorophenoxyacetic acid	µg/L	-32.22	-0.17	0.08	0.036	*
Deet Acid	µg/L	-43.23	-0.25	0.14	0.088	
Oxychlordane	ng/g	-51.57	-0.31	0.05	6.43×10^{-7}	***
Trans-nonachlor	ng/g	-51.61	-0.32	0.05	1.22×10^{-7}	***

Significance codes: '***' 0.001 '**' 0.01 '*' 0.05

These models are comparing farmworkers and non-farmworker pesticide exposure by the pesticide of interest. Each model in this table is as follows: chemical concentration =intercept+ β worker category+ β_2 urinary creatinine (used for non-persistent chemicals). Using this model, β estimate and β standard error are presented in the above table. Farmwork is defined as 1=history of farmwork and 0=no history of farmwork.

The coefficient estimates and standard error for farmworker category are presented in the above table.

Since $\log_{10}$ transformation of the chemical concentration was completed prior to creating the model, percent change was created by exponentiating 10 to the chemical Beta estimate, subtracting 1 and multiplying by 100.

Table 15 Adjusted linear regression coefficients of citizenship variable when predicting chemical concentration

Chemical Name	*Units*	*Percent Change*	*Beta Estimate*	*Standard Error*	*p-value*	
2,5-Dichlorophenol	µg/L	469.71	0.76	0.26	0.029	*
p,p'-DDT	ng/g	150.23	0.40	0.09	3.0×10^{-4}	***
2,4-Dichlorophenol	µg/L	134.31	0.37	0.19	0.101	
p,p'-DDE	ng/g	61.32	0.21	0.11	0.080	
3-Phenoxybenzoic acid	µg/L	33.59	0.13	0.12	0.308	
3,5,6-Trichloropyridinol	µg/L	9.01	0.04	0.10	0.723	
Heptachlor Epoxide	ng/g	8.91	0.04	0.10	0.700	
Beta-hexachlorocyclohexane	ng/g	4.16	0.02	0.13	0.889	
Dieldrin	ng/g	3.31	0.01	0.05	0.796	
Oxychlordane	ng/g	2.28	0.01	0.11	0.927	
2,4-Dichlorophenoxyacetic acid	µg/L	-10.95	-0.05	0.15	0.736	
Trans-nonachlor	ng/g	-12.06	-0.06	0.08	0.494	
4-Nitrophenol	µg/L	-19.05	-0.09	0.12	0.462	
DEET acid	µg/L	-41.09	-0.23	0.14	0.150	

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

These models are comparing farmworkers and non-farmworker pesticide exposure by the pesticide of interest. This model is adjusted for citizenship status, body mass index, age, gender, racial ethnicity, survey year, creatinine or lipid, federal poverty-income ratio, and education level.

Farmwork is defined as 1=history of farmwork and 0=no history of farmwork.

The coefficient estimates and standard error for farmworker category are presented in the above table.

Since $\log_{10}$ transformation of the chemical concentration was completed prior to creating the model, percent change was created by exponentiating 10 to the chemical Beta estimate, subtracting 1 and multiplying by 100.

Chapter 4 Combining NHANES and Toxicity Forecast Dashboard Data to Compare Pesticide Exposure and Bioactivity, by Farmwork History and U.S. Citizenship

4.1 Introduction

Pesticides were first used in the United States (US) in 1930, and were commonly used across the US by 1950 [150]. From 1990 to 2018, pesticide use worldwide has steadily increased, with China, the US, and Brazil being the top three countries that use the most pesticides[150]. Based on 2017 data, the US imports one billion pounds and counting each year, and more than two billion people worldwide interact with pesticides annually [150]. In understanding the health effects of pesticides, epidemiologists and toxicologists have focused on quantifying pesticides in human tissue and the health disorders associated with the pesticide exposure or assessing the effects of pesticides *in vivo* or *in vivo*.

Persistent pesticides last in the environment and human body for years or even decades and can bioaccumulate. Persistent pesticides include organochlorines, dichlorodiphenyltrichlorethane (DDT), Lindane, Chlordane, Dieldrin, Heptachlor and their metabolites. Non-persistent pesticides include organophosphates, carbamates, pyrethroids, chlorinated phenols, acyl alanine fungicides and more chemical groups, and were thought to be the less harmful answer to previously used persistent chemicals (e.g. organochlorines) [133]. However, non-persistent chemicals still affect human health. While pesticides are associated with endocrine disruption, cancers, and motor neuron disorders, there is still a lack of human health research on the dose-response curve, toxicological mechanism or how population exposure concentrations relate to social determinants of health [1,44,67–70].

Pesticides, especially persistent chemicals like organochlorines, have been associated with endocrine disruption metabolism, puberty, and birth defects [7–10]. In a study of pregnant women in Puerto Rico, women exposed to glyphosate (OR=1.35, 95% CI: 0.99, 1.83) and its metabolite, Aminomethylphosphonic acid (OR=1.67, 95% CI: 1.26, 2.20) more often experienced spontaneous preterm birth, especially if these chemicals were detectable at 26 weeks of gestation [61]. In another study, infants exposed to 2,4-Dichlorophenol (24DCP) had slower auditory responses at 6 weeks, suggesting their hearing was impaired [151]. Specifically, 6-week old infants with high prenatal 24DCP exposures (>1.17ng/mL) had wave V latencies 0.12ms slower than infants without 24DCP exposure (p=0.01) based on auditory brainstem response [151]. In another study looking at prenatal exposure in California of 9,300 daughters over 54 years, mothers with the highest o,p-DDT concentrations were 3.7 times more likely to have daughters who developed cancer by the age of 52 in comparison to mothers with the lowest o,p-DDT blood concentrations[51]. In a French study, farmworkers were found to have more risk of developing a motor neuron disease (RR=1.13, 95%CI: 0.97,1.31) and Parkinson's Disorder (RR=1.10, 95%CI: 1.02, 1.18) than people who were not [73]. Pesticides currently in use have been associated with a multitude of adverse health outcomes in human populations. Novel strategies are needed to identify and predict pesticides with the highest risk of adverse health outcomes.

In 2008, the US EPA launched the National Toxicology Program and collaborated with multiple other federal agencies including the Food and Drug Administration and the National Institute of Environmental Health Sciences to create the Toxicology in the 21st Century (Tox21) program [19]. Through Tox21 researchers have been tasked with developing rapid testing methods, to determine the safety of chemicals such as food additives and pesticides. The aims of Tox21are to understand the biological mechanisms that chemicals alter, to create a prioritization for chemicals to be tested and to create a wealth of data that can more accurately predict *in vivo* toxicological responses in the human body [19]. There are currently 85,000 chemicals on the global market that TSCA has listed in its inventory of substances, and there is little to no experimental toxicology or epidemiology data on many of them [20].

The aim of this study is to determine if people residing in the US are exposed to bioactive concentrations of pesticides. Based on our results from Chapter 3, we hypothesize people residing in the US will be exposed to bioactive concentrations of pesticides. Moreover, we hypothesize farmworkers will be exposed to bioactive concentrations of pesticides more frequently than non-farmworkers. This project will test these hypotheses by comparing the concentrations distributions of chemicals in NHANES with the bioactivity distributions of those chemicals in Toxcast. In addition, our project will quantify which cellular target families are most often affected by these pesticides, and look to see how these target families differ by history of farmwork and U.S. citizenship status.

4.2 Methods

4.2.1 The National Health and Nutrition Examination Survey (NHANES)

NHANES is a cross-sectional study representative of the US population that examines the health and nutrition of adults and children residing within the US. The survey captures substantial information on the respondents including as self-reported occupation, and demographics such as age, gender, citizenship, poverty-income ratio, and education. For many participants, urinary and blood concentrations of pesticides and pesticide metabolites are also quantified.

This study retained all NHANES study participants aged 18 years and older who also have occupation and pesticide exposure data present between 1999 and 2014. All data was downloaded using the RNHANES package and included the demographic data. Using the current industry (OCD231=1 and OCD230=1), current occupation (OCD241=18 and OCD240=18), longest industry (OCD391=1 and OCD390=1), and longest occupation (OCD392=18), everyone who put "Agriculture, Forestry and Fishing" was coded as a farmworker.

All urinary biomarker measurements were adjusted for urinary creatinine, and all blood pesticide biomarker measurements were blood lipid adjusted. Detectability percentages were calculated by dividing the total number of measurements above LOD by the total number of the chemical's measurements in NHANES. To ensure that we included chemicals with values above the limit of detection in the majority of the study participants, detectability percentages of 50% and higher were maintained which resulted in 14 chemicals of interest [39].

These chemicals included the following: 2,4-Dichlorophenol (24DCP), 2,4-Dichlorophenoxyacetic acid (24D acid), 2,5-Dichlorophenol (25DCP), 3,5,6-Trichloropyridinol (TCP), 4-Nitrophenol, β-hexachlorocyclohexane (β-HCH), diethyltoluamide acid (DEET acid), Dieldrin, Heptachlor Epoxide, 3-phenoxybenzoic acid (3-PBA), p,p'-DDE, and p,p'-DDT. Additionally, the measurements of TCP, a chlorpyrifos metabolite, were compared to the Toxcast toxicity data for both CPF and chlorpyrifos-oxon (CPO).

4.2.2 Calculating Molarity for NHANES measurements

Chemical doses used in Toxcast assays are reported in molarity and the chemical biomarker measurements in NHANES are in ng/µL (for urine samples) or ng/g (for lipid adjusted serum samples). To be able to make comparisons between these two datasets, the NHANES measurements were converted to molarity by dividing the chemical measurement by the molecular weight of the chemical. Since the molecular weight and CAS registry number (CASRN) are not provided in NHANES, we used the chemical names provided in the NHANES codebook to search the National Center for Biotechnology and Information's PubChem Library. PubChem contains the largest collection of open access chemical information that is commonly found on the Material Safety Data Sheets (MSDS) as well as resources with pertinent information on the toxicity, patents, and other topics of each chemical. From this search we collected each chemical's PubChem ID, CASRN, common name, and molecular weight. Toxcast could then be queried for the CASRNs of the chemicals present in NHANES.

4.2.3 Toxicity Forecast Dashboard Data

The US EPA's Toxicity Forecast Dashboard (Toxcast) is a collection of publicly available high throughput toxicity data intended to make chemical assessment more accessible by allowing researchers to search which chemicals show toxicological effects more easily within human tissue. High throughput toxicity screening initiatives have been developed to quantify biological effects of pesticides *in vitro*. Dose response curves are created for each chemical and assay, and from these curves the activation concentrations and positive hitcalls are defined. ACC is the concentration at which the model reaches the cut-off values for the data-series to be considered active and is based on the levels of significance for the dose curve response. The ACC can be used as a proxy of potency to determine the genes, proteins, enzymes, effects on biological pathway and viabilities at which chemicals are active.

Assay data for the 15 pesticides from NHANES were then extracted from the Toxcast database. We retrieved the hitcall (representative of an active assay), the activity concentration at cutoff (or ACC), and the intended target family of each Toxcast assay based on the 16 pesticides from NHANES. Using the hitcall variable, we labeled assays as positive (hitcall==1) or negative (hitcall==0) to mean that an assay did or did not show bioactivity by the pesticide. We created a bioactivity ratio per chemical by dividing the number of positive assays by total number of assays. All 15 chemicals in NHANES were present in Toxcast. However, Trans-nonachlor was not maintained in the study because there were only 8 completed assays in Toxcast and none of those assays were active.

4.2.4 Data Management and Analysis

All data management and analysis were completed in R 4.0.5 GUI 1.74 Catalina build. Graphics were created using the *ggplot2* package library[153]. All NHANES data was downloaded using the RNHANES packaged in R[53]. The main outcomes of this project include 1) quantifying the distribution of the pesticide concentrations across NHANES and Toxcast, 2) quantifying the demographics of people with and without bioactive measurements, and 3) investigating how bioactivity differs by chemical, farmwork history, and US citizenship status. These outcomes will inform the overarching project question of whether people residing in the US are exposed to bioactive levels of pesticides, how these bioactive pesticides affect the body, and whether the rates of exposure to bioactive pesticide concentrations vary based on sociodemographic factors.

4.2.5 Understanding Pesticide Toxicological Mechanism Similarities

Using the *UpSetR* package, UpSet plots were created to better compare and contrast the similarities and differences in positive assays for each chemical in Toxcast[154]. The set size presents how many positive assays were identified for each chemical, the intersection histogram provides the count of similar assays between each chemical, and the dot-line plot to the bottom directs us to which chemicals are included in each intersection bin. By understanding what overlap exists between positive assays in Toxcast among pesticides, our goal is to better understand which biological targets are most likely affected in the human body by pesticide exposure. In the discussion, we hope to explore how known pesticide mechanisms of action may act upon these intended target families within human tissue.

4.2.6 Comparing NHANES and Toxcast

Using the corresponding Chemical Abstracts Service Registry Numbers (CASRNs) obtained from PubChem, Toxcast was matched to NHANES. From this new dataset, we created pesticide concentration distribution boxplots by the chemical and farmwork history or U.S. citizenship. These boxplots were created in the *tidyverse* using the *ggplot2* R package [59,60]. Pesticide distributions were overlaid unto the same axis to quantify overlap between the pesticide concentration distributions of exposure in NHANES participants and bioactivity in Toxcast.

For understanding the distribution of exposure in comparison to pesticide bioactivity concentrations, Toxcast and NHANES were combined using the Molar pesticide concentrations on the same axis by using the pesticide CASRNs. Using the *ggplot2* and *tidyverse* R packages, the boxplots of these concentration distributions were overlayed unto the same axis to directly compare the concentrations of pesticides that NHANES participants are exposed to with the concentrations of bioactive pesticides in Toxcast.

Next, we labeled anyone who had at least one chemical measurement equal to or above the minimum Toxcast ACC for that chemical as being "bioactive". Anyone who did not fit this group was defined as "non-bioactive." Demographics were quantified by bioactivity status among all study participants and then among farmworkers only. For continuous variables like body mass index (BMI) or age in years, we present the mean and standard error, and for all categorical variables, the stratified frequencies and sub-group percentages are provided. Differences in demographic factors by group or citizenship were tested using a Pearson's chi-square test, using a Rao and Scott Adjustment where necessary for categorical variables. Low response was defined as 8 or less respondents within one stratum. And for continuous variables, a Wilcoxon Rank test was used to test group means, with a Kruskall-Wallis Correction. All significance testing was completed using the NHANES Full Sample 2 and 4 Year MEC Exam Weights. A new weight variable titled "MEC16YR" was created using the weighted MEC 2 and 4 year measurements to represent the weights used from 1999-2002 and each year after, respectively.

Next, we calculated bioactivity by the chemical and marked measurements as bioactive based on their hitcall equaling 1. For model outcomes this bioactivity status by chemical was used as the outcome variable for logistic regression models used to investigate how the odds of being a farmworker and having at least one bioactive measurement differ from non-farmworkers by the chemical. These models were adjusted for BMI, age, poverty-income ratio (PIR), survey year, gender, racial ethnicity, U.S. citizenship status, farmwork history, country of birth and education level. After comparing all study respondents' odds of having a bioactive measure, we created logistic regression models comparing U.S. citizenship status. These models were also adjusted for BMI, age, PIR, survey year, gender, racial ethnicity, country of birth, and education level.

4.3 Results

To better understand how each of the chemicals relate to each other, Appendix A has been included with an outline of the chemicals by persistence and chemical category. In total, there are 14 pesticides that are detectable in NHANES study participants and also assayed in Toxcast. Overall, there were 6 persistent organic pesticides and 8 non-persistent pesticides included in this study. Table 16 presents all the chemicals assessed in this study and the number of assays ran within Toxcast. The top three most bioactive pesticides in Toxcast were heptachlor epoxide had the highest percentage of assays which were "active" (39.85%), followed by p,p'-DDT (35.73%) and p,p'-DDE (26.78%).

Figure 5 presents the upset plot comparing the overlap in active assays of the 14 pesticides of interest. For example , p,p'-DDE, p,p'-DDT, and heptachlor epoxide have the most active assays in common with 25 overlapping assays. CPF, dieldrin, CPO, p,p'-DDE, p,p'-DDT, and heptachlor epoxide had 14 active assays in common. Overall, p,p'-DDE has the most unique active assays with 30 assays, and DEET acid had the least with only 2 positive assays that no other chemical had as well. These assays are being used as a proxy of the biological mechanisms affected by the chemicals and can identify what similarities or differences may exist in biological targets for the different pesticides.

Table 17 presents the model ACC's for positive Toxcast assays per chemical ranked by the count of chemicals positive for that assay. Overall, the top 3 assays which are commonly activated by the pesticides in NHANES were Attagene's gene expression assay for pregnane-X-receptor (PXR) target genes (PXRE_CIS_up) (N=11) and TOX21's mitochondrial membrane potential assays (MMP_ratio_down (N=11) and TOX21's MMP_rhodamine (N=10), another mitochondrial membrane potential assay. PXR is a nuclear receptor used in the identification of xenobiotics, and in more recent research evidence exists that PXR may also regulate genes implicated in glucose, lipid, and bile acid metabolism [155]. The chemical with the lowest model ACC for the PXR target gene assay was ß-hexachlorocyclohexane (0.96µM) and the highest was 2,4-Dichlorophenol (6.7µM). For MMP, Dieldrin had the lowest model ACC (4.02µM), while chlorpyrifos had the highest (36.08µM).

When trying to understand what intended target families are most affected by these chemicals, Table 18 provides the frequency of intended target families by the pesticide. In Table 18, cell cycle (N=487), nuclear receptor (N=318), cytokine (N=143), DNA binding (N=172), and cell adhesion molecules (N=65) were the most frequently intended targets of the pesticides included in the study. Overall, p,p'-DDE (N=305) had the most intended target family counts based on positive assays, followed by p,p'-DDT (N=278), heptachlor epoxide (N=259), and chlorpyrifos (N=126). Heptachlor epoxide had the highest number of positive assays targeting the cell cycle (N=123) and p,p'-DDT had the second most (N=120). Additionally, for p,p'-DDE had mostly nuclear receptor targeting positive assays (N=102), followed by the cell cycle (N=74) and DNA binding (N=64).

Next, we wanted to compare the concentrations of chemicals required to activate the Toxcast assays to the levels measured in people in NHANES. Figure 6 presents the distribution of pesticide concentrations among people residing in the United States in orange (retrieved from NHANES), and in blue, the concentration of chemicals of ACC (retrieved from Toxcast). In this figure, where the pesticide distributions of exposure and bioactivity overlap represents a pesticide exposure among the US population that is “bioactive”. Additionally, 4-nitrophenol is the only pesticide biomarker in NHANES that does not have human measurements that overlap with the bioactive distribution in NHANES. Moreover, NHANES participants had biomarker concentrations of heptachlor epoxide, chlorpyrifos, 24D acid , 2,4-D, and 3-PBA high enough for bioactivity in at least one Toxcast assays. All of the remaining chemicals also overlapped with bioactive concentrations in Toxcast, with p,p’-DDE and β-HCH having median participant exposure measurements that overlap with the range of bioactive measurements.

The stratified demographics for people with and without at least one bioactive measurement within NHANES are presented in Table 19. Overall, people with bioactive measurements had a significantly lower mean federal poverty-income ratio (2.58 vs. 1.6, p= 5.82×10^{-6}). More than half of all farmworkers (58.22%) had bioactive measurements. Roughly half of all Mexican Americans (50.78%) had bioactive pesticide measurements, which was different from the only 33.04% of people who identified as “Other Hispanic” having bioactive measurements. Almost half of all non-Hispanic Black people (45.32%) had at least one bioactive pesticide measurement.

Table 20 presents the demographics of farmworkers within the NHANES by whether the farmworker has at least one bioactive measurement. In this table, people with bioactive pesticide measurements had significantly lower mean PIR (2.58 vs 1.66, p=1.68×10^{-16}). Additionally, survey year, citizenship status, country of birth, and education level make ups significantly differed based on bioactivity. The majority of all people with bioactive measurements were recruited between 1999 and 2004 (N=538, 81.3%). The majority of people born in Mexico (N=88, 87.13%) had at least one bioactive measurement.

When looking at overall bioactivity by person (not included in tables and figures), we found farmworkers were 1.37 times more likely to have a bioactive measurement in comparison to non-farmworkers (unadjusted OR= 1.75, 95% CI: 1.44, 2.14; adjusted OR=1.37, 95% CI: 1.10, 1.71). Next, we narrowed to farmworkers only and found farmworkers living without U.S. citizenships were 1.31 times more likely to have a bioactive measurement compared to farmworkers with U.S. citizenship (unadjusted OR=1.01, 95%CI: 0.67,1.52; adjusted OR 1.31, 95% CI: 0.75, 2.30), however, this relationship was not statistically significant.

Next, we investigated how the odds of having a bioactive concentration of an individual pesticides differed by farmwork history. The unadjusted results for these regressions are presented in Table 21. In the unadjusted regressions we see that 24D acid , DEET acid, 3-PBA, 4-nitrophenol, TCP, CPF and CPO significantly differed by farmwork history. However, after adjusting for social determinants of health and other confounders in Table 22, farmworkers were 1.69 times more likely to have a bioactive measurement of DEET acid in comparison to non-farmworkers (OR=1.69, 95% CI: 1.24, 2.32, $p = 6.4 \times 10^{-3}$).

We then narrowed the study population to farmworkers only to understand the odds of having a bioactive measurement for a given pesticide by citizenship status. Table 23 presents the unadjusted results, and every pesticide has significantly higher odds of a bioactive measurement among non-citizen than citizen farmworkers, except for DEET acid. After controlling for BMI, age, gender, racial ethnicity, survey year, PIR, education level, and urinary creatinine or lipids, as shown in Table 24, β-HCH, Dieldrin, Heptachlor epoxide, p,p'-DDE, p,p'-DDT, 24D acid , 3-PBA, 4-nitrophenol, TCP, CPF, and CPO farmworkers living without US citizenship were significantly more likely to have a bioactive level of these pesticides in comparison to U.S. citizens. For example, as shown in Table 24, farmworkers without U.S. citizenship were 1.49 times more likely to have a bioactive measurement of Heptachlor epoxide (OR=1.49, 95% CI: 1.35, 1.66) or p,p'-DDT (OR=1.49, 95%CI: 1.34, 1.65). Additionally, non-citizens were 1.46 times more likely to have a bioactive measurement of Dieldrin (OR=1.46, 95% CI: 1.30, 1.63).

4.4 Discussion

Farmworkers have significantly different health outcomes when compared to non-farmworkers[135–137,156–158]. Researchers have also identified marked increase in cancers, endocrine disruption, and neuronal disorders in migrant workers and other farmworkers living without citizenship in the U.S. [129,137,157,159,160]. These disease rates are also increased for people exposed to pesticides, both persistent and non-persistent. Social determinants of health like PIR, gender, and citizenship can further increase the divide in healthcare utilization, treatment, and overall health[17,36,140,161]. By further understanding the mechanisms behind pesticide active ingredients and bioactivity, our study investigates the possible connection between social determinants of health and pesticide exposure. More specifically, we looked at history of farmworker and working in the U.S. without citizenship status have on exposure to bioactive concentrations of pesticides as determined by high throughput toxicity screening.

When looking at individuals who have pesticide biomarker concentrations at these bioactive levels, demographics statistically differed based on bioactivity, farmwork history and citizenship status. We found NHANES participants are exposed to bioactive concentrations of pesticides. This study includes 14 chemicals with 6 persistent organic pesticides and 8 non-persistent pesticides that are included in both NHANES and Toxcast. Heptachlor epoxide, p,p'-DDT, and p,p'-DDE were the most bioactive pesticides in Toxcast based on overall percent of positive assays. Additionally, CPF, Dieldrin, CPO, p,p'-DDE, p,p'-DDT, and Heptachlor epoxide overlapped on 14 assays suggesting similar mechanisms among these chemicals.

4.4.1 Pesticide Exposure and Health.

Pesticides have been associated with chronic health conditions like diabetes mellitus, obesity, and different types of cancers[10,71,75,159,160,162]. Pesticides have also been associated with increased mortality due to cancer, diabetes mellitus, poisonings, and tuberculosis and other lung infection [146,148]. Pesticide exposure throughout the life course has been associated with breast cancer and dysregulated mammary gland development. Overall, mothers with the highest o,p-DDT concentrations were 3.7 times more likely to have daughters who developed cancer by the age of 52 in comparison to mothers with the lowest o,p-DDT blood concentrations[51]. The association between CPF exposure and breast cancer has not been as clearly established, however there is research suggesting that CPF plays a role in breast cancer initiation and cell growth. CPF exposure increased breast cancer risk among women married to farmworkers (RR: 1.41, 95% CI (1.00,1.99)), and more specifically both estrogen dependent and independent breast cancer risk increased (RR: 1.37, RR: 2.26, respectively) [4]. CPF has also been linked to endocrine disruption in relation to mammary gland development and hormonal balance in adult rats[65].

Mechanisms of action for the pesticides in this study and the research on health outcomes could help to inform these differences in health outcomes and help researchers understand how these chemicals are directly harming the human body. Many of these insecticides' mechanism of action involves nervous system toxicity [9,29]. For example, organochlorines block normal γ-aminobutyric acid (GABA) protein function by attaching to the A complex of the ionosphere protein complex which results in an overaccumulation of chloride in the synaptic gap[9,29]. Thus, exposure to these chemicals, like DDT or even non-persistent pesticides such as 2,4-dichlorophenoxyacetic acid, can ultimately cause a decrease in central nervous function and glucose and lipid metabolism [9,29]. Organophosphates and carbamates inhibit acetylcholinesterase (AChE), the enzyme responsible for breaking down acetylcholine in the synaptic gap into acetate and choline[133]. When acetylcholine builds in the synaptic gap, it can cause an overstimulation of the neuron and lead to convulsions, tremors, paralysis and death [133]. While organophosphates permanently inhibit AChE, whereas carbamates reversibly inhibit AChE[133].

PXR was a common target for the chemicals included in this chapter. PXR was first identified in mice in 1998 and is activated by naturally occurring and synthetic pregnanes and glucocorticoids [163]. These results here also showed that PXR had some effect on cytochrome protein (CYP) 3A gene promoters [163]. By affecting CYP450 3A4, PXR affects the pathway for xenobiotic sensing and biotransformation essential to energy metabolism and decreases energy homeostasis related to toxicant metabolism [155]. Specifically, 14 conazole fungicides included in Toxcast's high throughput chemical risk assessment have also been found to cause PXR-related liver hypertrophy based on biological-pathway-altering concentration distribution [164,165]. One of these fungicides, Diflucan or fluconazole, is commonly prescribed to treat yeast infections due to candida in humans [166].

Another alteration common to pesticides in our study was changes to MMP, which has been associated with oxidative stress, genotoxicity, Parkinson's Disease, and metabolic disorders [1,167–170]. Specifically, Dieldrin, a chlorinated cyclodiene, targets mitochondria to cause cellular apoptosis [170]. In a study culturing human colon carcinoma cells an organophosphate, diazinon, was found to be cytotoxic because it causes oxidative damage by creating free radicals and inducing lipid peroxidation leading to DNA fragmentation [169]. Further research using Toxcast data also showed the mitochondria may be a target of organophosphate and carbamate pesticides in early life stages [167] Moreover, researchers have also investigated the effects of exposure to organochlorides has on the mouse liver and hepatic fatty acid content [168]. After an 8-week exposure to p,p'-DDE or ß-HCH in mice, it was clear that both chemicals accumulated in adipose tissue primarily and parenchymal organs, with liver being the secondary site of accumulation [168]. This study also found decreased expression of genes involved in mitochondrial fatty acid ß-oxidation and impairment of mitochondrial function [168]. The results of these studies suggest that pesticide exposure is associated with multiple mechanisms of harm in the human body besides just the mechanism of action for killing unwanted pests. These side effects of pesticide exposure can result in neuronal damage, cellular toxicity and death, and metabolic disorders through oxidative stress and gene and enzymatic alterations.

4.4.2 Pesticide Exposure and Endocrine Disruption.

Pesticides, such as organophosphates and organochlorines, are also endocrine disruptors that can increase cell proliferation, upregulate and downregulate hormones and their receptors[55,65,129,171,172]. Using 40 day old female Sprague-Dawley rats exposed to CPF over 100days, CPF was found to alter mammary gland proliferation and hormonal balance[65]. Specifically, CPF was found to increase progesterone receptor in control versus exposed rats (12.2 ± 3.0% vs. 17.4 ± 6.0% of mammary cells are progesterone positive), and to increase proliferating cell nuclear antigen in epithelial duct cells (21.8% ± 3.3% vs. 4.7 ± 1.9%,

$p<0.001$)[65]. In another study comparing hormone responsive breast cancer cells and non-hormonal cells that have been exposed to chlorpyrifos, cell proliferation was supported through estrogen receptor positive cells in MCF-7 and also lead to oxidative stress in MCF-7 and MDA-MB-231[55].

Quantifying chemical mixtures across a population is complex and methodology for understanding these mixtures is still novel. Researchers have also found similar cell proliferation increases in human cell lines exposed to organochlorines (MCF-7, MDAMB231, and CV-1 cells) due to CPF exposure[171,172]. Using a mixture of organochlorine, MCF-7 cells had an on average 2.4-fold increase in proliferation with the largest effect seen being a 3.2-fold increase at the $20x10^3$ concentration ($p<0.001$)[171]. Additionally, in the absence of hormones, each DDT compound mixture significantly increased cell proliferation in estrogen and dihydrotestosterone hormone responsive cells[171]. Specifically, p,p'-DDT had a 40% increase ($p<0.05$) while p,p'-DDE had an increase of 90% in cell proliferation in the absence of hormones ($p<0.05$) [171]. However, there is still plenty of research to be done in understanding chemical mixtures.

In the Hispanic Community Health Study/Study of Latinos, a cross-sectional study of 7,404 employees between the ages of 18 and 74 years (54% men), 4.7% of all employees reported using pesticides in the current occupations [72]. Among individuals who reported working with pesticides, the prevalence of any cardiovascular disease was 2.18 (95% CI: 1.34, 3.55), and specifically, atrial fibrillation (5.92, 95% CI: 1.89, 18.61) and coronary heart disease (2.20, 95% CI: 1.31, 3.71) had the two highest prevalence ratios [72].

People with bioactive pesticide biomarker measurements had significantly lower mean PIR compared to people without at least one bioactive biomarker measurement (2.42 vs 2.58, $p=1.68x10^{-16}$). Additionally, over half of Mexican Americans and almost half of non-Hispanic

Black Americans had bioactive measurements. Farmworkers without U.S. citizenship were significantly 1.49 times more likely to have a bioactive measurement of Heptachlor epoxide or p,p'-DDT. Overall, our study has found human exposure overlaps with known bioactive concentrations for pesticide exposure based on a combination of NHANES and Toxcast data.

4.4.3 Limitations and Strengths

A large limitation of this study is that not every chemical is measured in every participant, and that not every assay is completed in each chemical. This limitation makes direct comparisons impossible and therefore our results are somewhat limited to group means. There are some known limitations to the Toxcast dataset such as interference of cytotoxicity. Non-specific cell stress can interfere with the frequency reading since the cell is overworking to re-gain homeostasis after chemical exposure. Currently Toxcast primarily has chemicals that are soluble in ethanol or dimethyl sulfoxide (DMSO) which means that many water insoluble chemicals are not currently included in the Toxcast database. Currently the assays completed in Toxcast do not require solubility, which make testing of water insoluble chemicals difficult to test. Toxcast assays are often assessing effects in a single tissue cell type, which may not accurately reflect chemical sensitivity across organ systems or within particularly susceptible individuals. Moreover, while Toxcast maintains a robust suite of assays measuring effects across a broad spectrum of potential toxic outcomes, not every chemical is tested for every assay and not all potential biological outcomes following chemical exposure are captured.

Other limitations inherent to interpreting bioactivity also exist. For starters, urine and serum concentrations directly correlate with excreted or circulating concentrations, respectively, and therefore may not be representative of concentrations in target organs like fat, liver, kidneys, or brain. This is important because many chemicals target specific organs (e.g., organochlorines targeting the central nervous system) or bioaccumulate in specific tissue types like lipids. However, serum and urine will not capture these quantities of chemicals. Additionally, when plating cells with these chemicals it is important to understand that the chemicals may also enter the matrix of the assay which can cause misreads or alterations in exposure and growing patterns. Furthermore, some chemicals can be volatile and difficult to stabilize, can bind to the plastic culture plate, and could be active at the surface which can be difficult due to preferential adsorption. There are also challenges to being able to relate metabolites to their parent compounds since some chemicals can have more than one parent compound (e.g. the pyrethroid metabolite 3PBA). This can make ascertaining what active ingredient is bioactive in the human body difficult, and even if considering a limited number of chemicals, there is no way to calculate a direct contribution of each parent compound to a non-specific metabolite.

This is the first study comparing human exposure concentration distribution in NHANES to the distribution of bioactive chemical concentrations in Toxcast. This is a very important comparison to quantify to be able to determine clinically relevant dosing of toxicological experiments. Additionally, this project can inform evidence-based guidelines and policies that are focused on reducing pesticide exposure concentrations among people residing within the United States. Toxcast & NHANES are both validated, reliable study datasets created by the US government to assess chemical bioactivity and examine the health of people residing in the US. By integrating these two datasets, the results are more generalizable to the U.S. population.

4.4.4 Future Directions for Research.

While NHANES quantifies a large number of chemical biomarker concentrations for each study participant, these measures do not fully capture how many chemicals each person may be exposed to since every chemical is not tested for in every person. Moreover, toxicological research should continue to focus on novel methods for assessing toxicity of chemical mixtures and interactions to better understand population pesticide exposure and bioactivity of combined pesticide exposures in at-risk individuals. Currently, research looks at predominantly the active ingredients of pesticides, but inactive ingredients used to create pesticides may also influence human health that is currently being missed within the literature. Future research should include temporal data on pesticide exposure. Both NHANES and Toxcast include singular exposure time points in humans and in human cells, respectively. However, for many farmworkers, pesticide exposure is chronic and happens over multiple exposure incidents.

Additionally, it is possible that the occupational status of farmworkers and non-farmworkers are misclassified in NHANES, biasing our results toward the null. Specifically, we included all people with a current or previous occupation or industry response that included "Agriculture, Forestry, and Fishing," which means that some people could be miscategorized. We also did not assess the professions across the non-farmworkers which means that we could have possibly missed some people whose occupations also require pesticide exposure such as pesticide manufacturing or lawncare.

An important future direction would be to include data from the Agriculture Health Study or another farmworker-specific study that includes chemical biomarker measurements in the bioactivity analysis with Toxcast. By comparing the Toxcast bioactivity to data that is intended for understanding chemical exposures in farmworkers, we expect to have more accurate classification of occupation and more occupation-specific chemical biomarkers to compare to bioactivity concentrations. Additionally, we may find that farmworkers in these agriculture-specific studies are more highly exposed to pesticides than the NHANES study participants.

Expanding the health outcomes of interest under study and using prediction to look at health disorder symptomology would also be an important area of future research. This way we can better connect target families to health outcomes and stratify by occupation and social determinants of health like income, gender, citizenship, and country of birth. In this same vein of understanding social determinant effects on health, more research on how these biomarker concentration distributions differ based on residing or working in a low versus high income country because region and laws within a nation can alter the health and exposure for many. Ultimately, we would also like to include the reference values for many of these pesticide biomarkers to determine if the likelihood of being outside of the guidelines differs by social determinants of health.

4.5 Figures

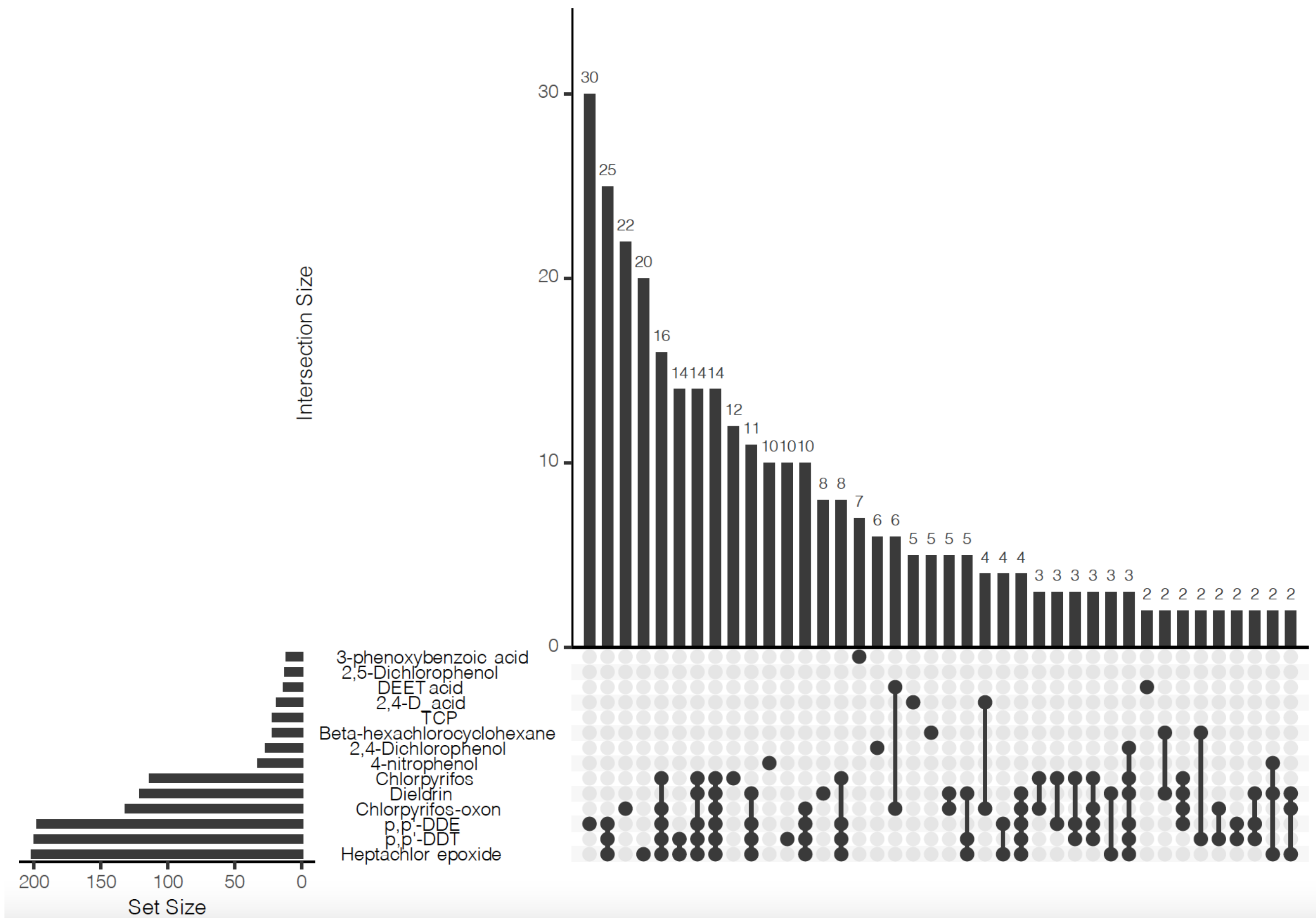

Figure 5 Upset Plot Quantifying and comparing Positive Assay in Toxcast, by the Pesticide

This figure was created using the UpSetR package. The histogram presents the count of assays that are similar among the pesticides highlighted by the dots at the bottom of the histogram. A positive assay was defined as having a positive hitcall. The data for this table was retrieved from the U.S. EPA's Toxicity Forecast Dashboard.

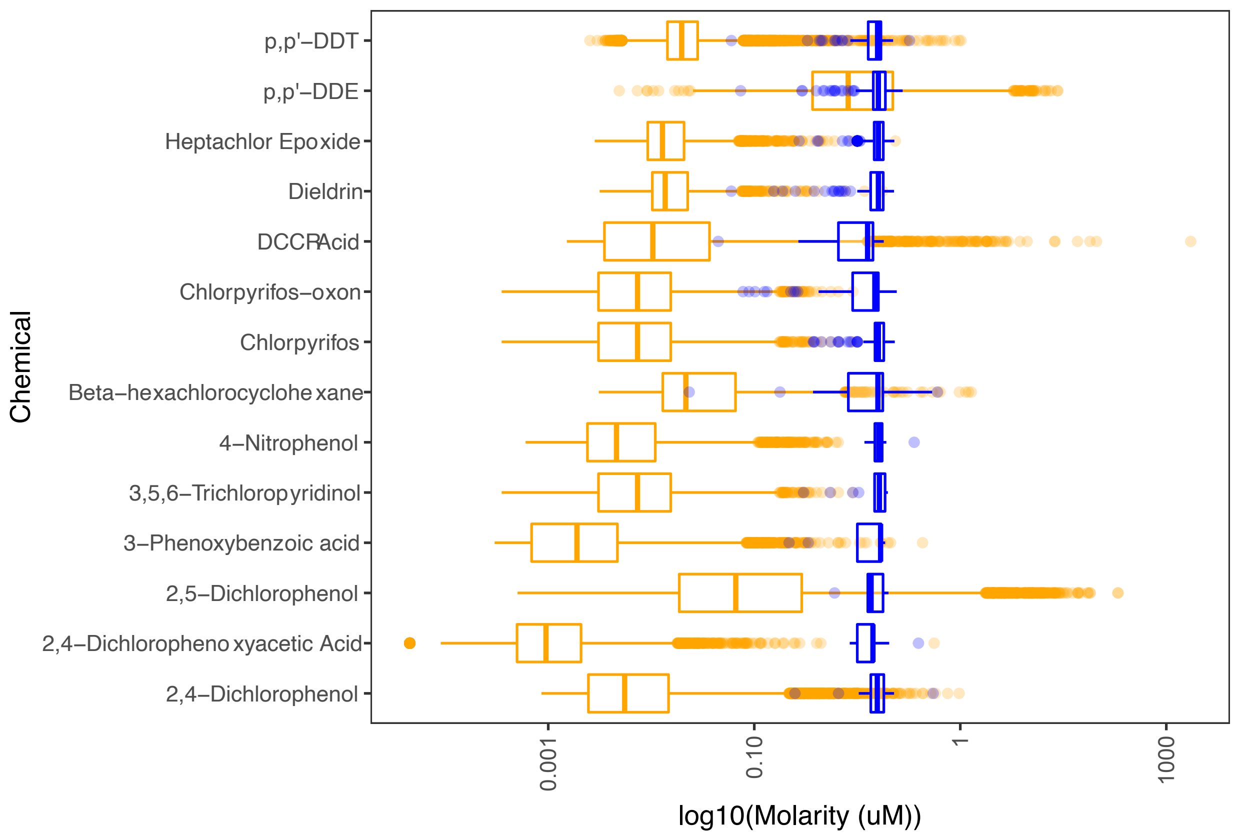

Figure 6 Distribution Comparisons between NHANES Participant Pesticide Exposure and Toxcast Bioactive Measurements, by the Pesticide

This figure presents pesticide distributions of exposure from NHANES and the positive assay concentration distributions in Toxcast, by the pesticide. NHANES data is presented in orange and represents human exposure, whereas Toxcast data is presented in blue and represents the concentrations of pesticides that have a known effect on the human cell. Bioactive was defined as having at least one pesticide biomarker concentration that was the same or higher concentration than the minimal concentration needed to see an effect. The data for this table was retrieved from the U.S. EPA's Toxicity Forecast Dashboard and the National Health and Nutrition Examination Survey.

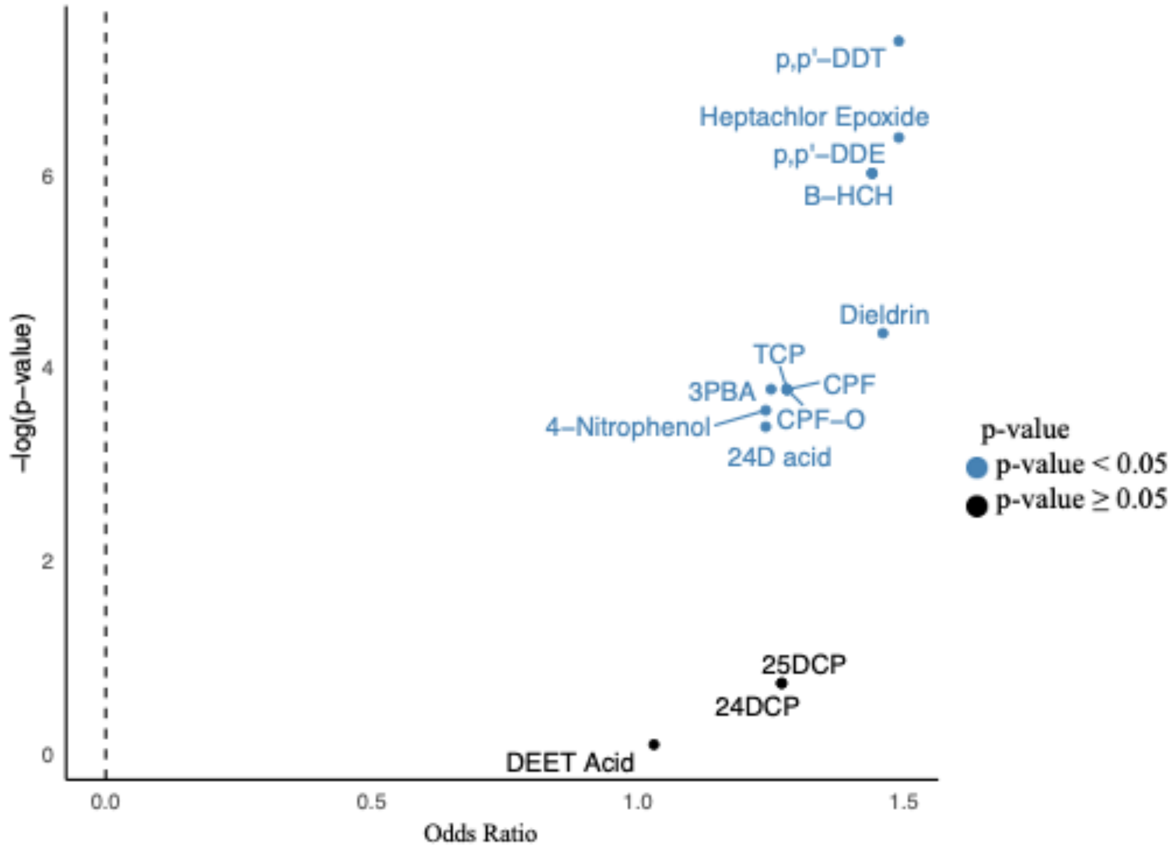

Figure 7 Volcano Plot of the Adjusted Logistic Regression Models of Pesticide Bioactivity among Farmworkers, by U.S. Citizenship Status

This figure presents the -log(p-values) and percent difference in bioactive pesticides between citizen and non-citizen farmworkers from our logistic regression model that is adjusted for citizenship status, country of birth, body mass index, age, gender, racial ethnicity, survey year, creatinine (urinary measurements) or lipid (blood measurements), poverty-income ratio, and education level. Bioactive was defined as having at least one pesticide biomarker concentration that was the same or higher concentration than the minimal concentration needed to see an effect. The data for this table was retrieved from the U.S. EPA's Toxicity Forecast Dashboard and the National Health and Nutrition Examination Survey.

4.6 Tables

Table 16 Bioactivity of pesticides cross-listed between NHANES and Toxcast, by pesticide and persistence

Common Name	*CAS-RN*	*Total Assays*	*Positive Assays*	*Bio-active Assay Percentage*	*Bioactivity Threshold (µM)*
2,4-Dichlorophenol	120-83-2	678	27	3.98	0.34
2,4-Dichlorophenoxyacetic acid	94-75-7	807	18	2.23	$6.49x10^{-3}$
2,5-Dichlorophenol	583-78-8	599	14	2.34	0.33
3-Phenoxybenzoic acid	3739-38-6	622	11	1.77	0.23
3,5,6-Trichloropyridinol	6515-38-4	433	21	4.85	1.35
4-Nitrophenol	100-02-7	682	43	6.30	$8.63x10^{-3}$
ß-hexachlorocyclohexane [a]	319-85-7	654	24	3.67	0.03
Chlorpyrifos	2921-88-2	639	126	19.72	1.45
Chlorpyrifos-oxon	5598-15-2	693	132	19.05	0.04
DEET Acid	134-62-3	1025	13	1.27	0.17
Dieldrin [a]	60-57-1	549	121	22.04	0.32
Heptachlor Epoxide [a]	76-44-8	650	259	39.85	1.31
p,p'-DDE [a]	72-55-9	1139	305	26.78	0.31
p,p'-DDT [a]	50-29-3	778	278	35.73	0.43

[a] Persistent Organic Pollutant.

A positive assay is defined as hitcall==1. The bioactivity assay percentage is created by dividing the total number of positive assays by the total number of assays and multiplying by 100%.

Bioactivity ratio per chemical was calculated by dividing the count of positive assays by the total number of assays within the US Environmental Protection Agency's Toxicity Forecast Dashboard database.

Table 17 Model Activation Concentration at Cutoff (uM) for Overlapping Toxicity Assays in Toxcast

Assay Name	Total Chemicals Active	p,p'-DDE	p,p'-DDT	3-PBA	ß-HCH
ATG_PXRE_CIS_up	11	4.318	1.389		0.962
TOX21_MMP_ratio_down	11	13.562	48.357		9.169
TOX21_MMP_rhodamine	10	23.881	48.608		
ACEA_AR_antagonist_80hr	9	2.671	2.791		4.825
ATG_ERE_CIS_up	9	9.793	1.062		0.873
NHEERL_ZF_144hpf_TERATOSCORE_up	9	2.077	1.588	5.401	
TOX21_RT_HEPG2_FLO_40hr_ctrl_viability	9	1.766	4.932	1.397	
ACEA_AR_antagonist_AUC_viability	8	2.776			4.878
TOX21_DT40_100	8	5.492	28.044		
TOX21_RT_HEK293_FLO_08hr_viability	8	5.603	4.864		0.230
ACEA_AR_agonist_AUC_viability	7	3.635	2.429		5.255
ATG_ERa_TRANS_up	7	4.869	0.951		0.782
ATG_NRF2_ARE_CIS_up	7	468.633	4.049		
BSK_LPS_CD40_down	7	4.963	2.718		

TOX21_DT40_657	7	6.087	8.534		
TOX21_PR_BLA_Antagonist_ch2	7	11.809	18.257		
TOX21_PR_BLA_Antagonist_ratio	7	5.007	5.790		
TOX21_TR_LUC_GH3_Antagonist	7	21.395	44.207		
TOX21_TR_LUC_GH3_Antagonist_viability	7	30.253	76.673		
ATG_MRE_CIS_up	6	7.208	7.217		
ATG_PPARg_TRANS_up	6			5.741	
ATG_PXR_TRANS_up	6	45.111	2.573		1.024
BSK_3C_HLADR_down	6	4.963	2.718		
BSK_3C_Proliferation_down	6	2.718	2.718		
BSK_3C_SRB_down	6	4.963	2.718		
BSK_3C_Vis_down	6	4.963	4.963		
BSK_4H_VCAM1_down	6	2.718	2.718		
BSK_hDFCGF_IP10_down	6	2.718	2.718		
BSK_hDFCGF_Proliferation_down	6	1.826	1.826		
BSK_LPS_VCAM1_down	6	4.963	4.963		
BSK_SAg_CD38_down	6	4.963	2.718		

BSK_SAg_CD40_down	6	4.963	2.718	
BSK_SAg_Proliferation_down	6	2.718	2.718	
BSK_SAg_SRB_down	6	4.963	2.718	
OT_FXR_FXRSRC1_0480	6	111.970	3.112	
TOX21_AP1_BLA_Agonist_ch1	6	18.623	64.140	
TOX21_ARE_BLA_agonist_ratio	6	6.917	6.542	
TOX21_CASP3_CHO_viability	6	6.558	32.275	
TOX21_DT40	6		19.354	
TOX21_ERb_BLA_Agonist_viability	6	41.163	27.090	
TOX21_ERb_BLA_Antagonist_ratio	6	12.927	9.526	
TOX21_PXR_viability	6	6.654	41.573	
TOX21_RT_HEK293_FLO_16hr_viability	6	5.230	4.728	
TOX21_RT_HEK293_FLO_24hr_viability	6	5.451	4.611	101.845
TOX21_RT_HEK293_FLO_32hr_viability	6	31.176	10.909	399.038
TOX21_RT_HEK293_GLO_08hr_viability	6	24.443	89.742	

TOX21_RT_HEK293_GLO_24hr_viability	6	26.601	85.830
TOX21_RT_HEK293_GLO_32hr_viability	6	28.817	88.817
TOX21_RT_HEPG2_FLO_24hr_viability	6	2.228	6.366
TOX21_RT_HEPG2_FLO_32hr_ctrl_viability	6	6.375	5.402
TOX21_VDR_BLA_Agonist_viability	6	4.407	4.063
UPITT_HCI_U2OS_AR_TIF2_Nucleoli_Antagonist	6	7.538	3.769

Assay Name	Total Chemicals Active	3,5,6-TCP	CPF-O	CPF
ATG_PXRE_CIS_up	11	4.474	3.889	1.458
TOX21_MMP_ratio_down	11	5.132	5.018	36.080
TOX21_MMP_rhodamine	10	5.271	5.333	6.995
ACEA_AR_antagonist_80hr	9		5.963	4.008
ATG_ERE_CIS_up	9	4.950	4.396	3.771

NHEERL_ZF_144hpf_TERATOSCORE_up	9		0.577	1.738
TOX21_RT_HEPG2_FLO_40hr_ctrl_viability	9		0.064	
ACEA_AR_antagonist_AUC_viability	8		4.062	3.956
TOX21_DT40_100	8	6.837	5.319	31.438
TOX21_RT_HEK293_FLO_08hr_viability	8			4.942
ACEA_AR_agonist_AUC_viability	7		3.942	4.787
ATG_ERa_TRANS_up	7		4.890	3.569
ATG_NRF2_ARE_CIS_up	7	7.256	2.766	3.928
BSK_LPS_CD40_down	7		4.963	4.963
TOX21_DT40_657	7		6.146	6.746
TOX21_PR_BLA_Antagonist_ch2	7		1.005	3.648
TOX21_PR_BLA_Antagonist_ratio	7		0.918	5.773
TOX21_TR_LUC_GH3_Antagonist	7	5.977	2.805	32.470
TOX21_TR_LUC_GH3_Antagonist_viability	7		4.490	38.152
ATG_MRE_CIS_up	6		6.747	6.500

ATG_PPARg_TRANS_up	6	2.809	3.660	5.688
ATG_PXR_TRANS_up	6			1.940
BSK_3C_HLADR_down	6		4.963	4.963
BSK_3C_Proliferation_down	6		4.963	4.963
BSK_3C_SRB_down	6		4.963	4.963
BSK_3C_Vis_down	6		2.718	4.963
BSK_4H_VCAM1_down	6		4.963	4.963
BSK_hDFCGF_IP10_down	6		4.963	2.718
BSK_hDFCGF_Proliferation_down	6		1.826	4.963
BSK_LPS_VCAM1_down	6		4.963	4.963
BSK_SAg_CD38_down	6		4.963	4.963
BSK_SAg_CD40_down	6		4.963	4.963
BSK_SAg_Proliferation_down	6		4.963	4.963
BSK_SAg_SRB_down	6		1.826	
OT_FXR_FXRSRC1_0480	6		4.632	4.526
TOX21_AP1_BLA_Agonist_ch1	6		3.322	6.912
TOX21_ARE_BLA_agonist_ratio	6	4.873		7.397

TOX21_CASP3_CHO_viability	6		6.487	
TOX21_DT40	6		6.191	32.334
TOX21_ERb_BLA_Agonist_viability	6	6.053	2.202	
TOX21_ERb_BLA_Antagonist_ratio	6	6.889	1.254	
TOX21_PXR_viability	6		6.128	
TOX21_RT_HEK293_FLO_16hr_viability	6			
TOX21_RT_HEK293_FLO_24hr_viability	6			
TOX21_RT_HEK293_FLO_32hr_viability	6			
TOX21_RT_HEK293_GLO_08hr_viability	6			6.261
TOX21_RT_HEK293_GLO_24hr_viability	6			7.259
TOX21_RT_HEK293_GLO_32hr_viability	6			8.067
TOX21_RT_HEPG2_FLO_24hr_viability	6		0.062	
TOX21_RT_HEPG2_FLO_32hr_ctrl_viability	6			
TOX21_VDR_BLA_Agonist_viability	6		2.009	5.990

UPITT_HCI_U2OS_AR_TIF2_Nucleoli_Antagonist	6		621.798	5.788

Assay Name	Total Chemicals Active	4-Nitrophenol	DEET Acid	Dieldrin	Heptachlor Epoxide
ATG_PXRE_CIS_up	11		3.575	1.062	2.052
TOX21_MMP_ratio_down	11	36.284		4.017	20.074
TOX21_MMP_rhodamine	10	39.101		4.519	26.644
ACEA_AR_antagonist_80hr	9	4.577		3.897	2.861
ATG_ERE_CIS_up	9			1.167	2.249
NHEERL_ZF_144hpf_TERATOSCORE_up	9	5.371		0.322	1.507
TOX21_RT_HEPG2_FLO_40hr_ctrl_viability	9	0.147		5.452	25.537
ACEA_AR_antagonist_AUC_viability	8	6.790		3.853	3.050
TOX21_DT40_100	8	5.256		3.540	27.444
TOX21_RT_HEK293_FLO_08hr_viability	8			5.234	17.180
ACEA_AR_agonist_AUC_viability	7			3.714	3.390
ATG_ERa_TRANS_up	7			2.149	

ATG_NRF2_ARE_CIS_up	7		7.320	
BSK_LPS_CD40_down	7		4.963	4.963
TOX21_DT40_657	7	4.782	2.760	15.907
TOX21_PR_BLA_Antagonist_ch2	7	2.769	3.395	34.961
TOX21_PR_BLA_Antagonist_ratio	7	0.012	1.977	9.645
TOX21_TR_LUC_GH3_Antagonist	7		4.671	14.795
TOX21_TR_LUC_GH3_Antagonist_viability	7	6.672	5.543	23.075
ATG_MRE_CIS_up	6		5.942	6.949
ATG_PPARg_TRANS_up	6		8.056	
ATG_PXR_TRANS_up	6		1.806	5.218
BSK_3C_HLADR_down	6		2.718	2.718
BSK_3C_Proliferation_down	6		4.963	2.718
BSK_3C_SRB_down	6		2.718	2.718
BSK_3C_Vis_down	6		2.718	4.963
BSK_4H_VCAM1_down	6		4.963	2.718
BSK_hDFCGF_IP10_down	6		4.963	2.718

BSK_hDFCGF_Proliferation_down	6			4.963	2.718
BSK_LPS_VCAM1_down	6			4.963	4.963
BSK_SAg_CD38_down	6			4.963	4.963
BSK_SAg_CD40_down	6			2.718	4.963
BSK_SAg_Proliferation_down	6			2.718	2.718
BSK_SAg_SRB_down	6		4.963	4.963	4.963
OT_FXR_FXRSRC1_0480	6			3.841	3.581
TOX21_AP1_BLA_Agonist_ch1	6			4.171	16.187
TOX21_ARE_BLA_agonist_ratio	6			5.210	22.543
TOX21_CASP3_CHO_viability	6	18.991		5.699	35.875
TOX21_DT40	6	11.153		3.005	26.007
TOX21_ERb_BLA_Agonist_viability	6			5.758	38.097
TOX21_ERb_BLA_Antagonist_ratio	6			3.760	28.847
TOX21_PXR_viability	6	22.327		6.796	46.548
TOX21_RT_HEK293_FLO_16hr_viability	6			4.923	17.316

TOX21_RT_HEK293_FLO_24hr_viability	6		4.528	15.807
TOX21_RT_HEK293_FLO_32hr_viability	6		4.395	15.337
TOX21_RT_HEK293_GLO_08hr_viability	6		4.358	18.247
TOX21_RT_HEK293_GLO_24hr_viability	6		4.456	17.812
TOX21_RT_HEK293_GLO_32hr_viability	6		4.663	19.293
TOX21_RT_HEPG2_FLO_24hr_viability	6		5.200	26.029
TOX21_RT_HEPG2_FLO_32hr_ctrl_viability	6	0.149	5.360	25.119
TOX21_VDR_BLA_Agonist_viability	6		4.658	22.065
UPITT_HCI_U2OS_AR_TIF2_Nucleoli_Antagonist	6		4.354	4.587

Assay Name	Total Chemicals Active	24D acid	25DCP	24DCP
ATG_PXRE_CIS_up	11		3.600	6.700
TOX21_MMP_ratio_down	11		14.544	17.037
TOX21_MMP_rhodamine	10		18.399	22.953
ACEA_AR_antagonist_80hr	9			6.040

ATG_ERE_CIS_up	9		5.940
NHEERL_ZF_144hpf_TERATOSCORE_up	9		4.849
TOX21_RT_HEPG2_FLO_40hr_ctrl_viability	9	22.606	6.538
ACEA_AR_antagonist_AUC_viability	8		6.147
TOX21_DT40_100	8		
TOX21_RT_HEK293_FLO_08hr_viability	8	0.330	0.352
ACEA_AR_agonist_AUC_viability	7		
ATG_ERa_TRANS_up	7		5.901
ATG_NRF2_ARE_CIS_up	7		4.032
BSK_LPS_CD40_down	7	1.826	
TOX21_DT40_657	7		
TOX21_PR_BLA_Antagonist_ch2	7		
TOX21_PR_BLA_Antagonist_ratio	7		
TOX21_TR_LUC_GH3_Antagonist	7		
TOX21_TR_LUC_GH3_Antagonist_viability	7		

ATG_MRE_CIS_up	6	
ATG_PPARg_TRANS_up	6	7.697
ATG_PXR_TRANS_up	6	
BSK_3C_HLADR_down	6	
BSK_3C_Proliferation_down	6	
BSK_3C_SRB_down	6	
BSK_3C_Vis_down	6	
BSK_4H_VCAM1_down	6	
BSK_hDFCGF_IP10_down	6	
BSK_hDFCGF_Proliferation_down	6	
BSK_LPS_VCAM1_down	6	
BSK_SAg_CD38_down	6	
BSK_SAg_CD40_down	6	
BSK_SAg_Proliferation_down	6	
BSK_SAg_SRB_down	6	
OT_FXR_FXRSRC1_0480	6	
TOX21_AP1_BLA_Agonist_ch1	6	

TOX21_ARE_BLA_agonist_ratio	6			
TOX21_CASP3_CHO_viability	6			
TOX21_DT40	6			
TOX21_ERb_BLA_Agonist_viability	6			
TOX21_ERb_BLA_Antagonist_ratio	6			
TOX21_PXR_viability	6			
TOX21_RT_HEK293_FLO_16hr_viability	6	4.217		0.335
TOX21_RT_HEK293_FLO_24hr_viability	6	4.132		
TOX21_RT_HEK293_FLO_32hr_viability	6	4.198		
TOX21_RT_HEK293_GLO_08hr_viability	6			3.925
TOX21_RT_HEK293_GLO_24hr_viability	6			2.813
TOX21_RT_HEK293_GLO_32hr_viability	6			234.755
TOX21_RT_HEPG2_FLO_24hr_viability	6		3.735	
TOX21_RT_HEPG2_FLO_32hr_ctrl_viability	6		3.556	

TOX21_VDR_BLA_Agonist_viability	6
UPITT_HCI_U2OS_AR_TIF2_Nucleoli_Antagonist	6

Table 18 Toxcast Assay Intended Target Family Frequencies, by Pesticide

Intended Target Family	2,4-D	24D Acid	2,5-D	3-PBA	3,5,6-TCP	4-Nitrophenol	Beta-HCH	Total
cell cycle	7	4	6	1	3	28	6	487
nuclear receptor	8	1	1	4	10	4	12	318
DNA binding	2	2	1	0	5	0	3	172
cytokine	0	2	1	0	1	0	0	143
cell adhesion molecules	0	0	0	0	0	0	0	65
cell morphology	2	0	2	0	1	2	1	38
CYP	0	6	0	0	0	0	1	32
GPCR	1	0	0	1	1	0	0	25
protease	0	0	0	1	0	1	0	19
malformation	1	0	0	1	0	1	0	12
steroid hormone	3	0	2	0	0	1	0	12
transporter	1	0	0	0	0	0	0	11
histones	0	0	0	0	0	0	0	9
oxidoreductase	1	0	0	0	0	3	0	8
phosphatase	1	0	0	2	0	1	0	7
protease inhibitor	0	0	0	0	0	0	0	7
esterase	0	0	0	1	0	0	0	6
growth factor	0	0	1	0	0	0	0	5
hydrolase	0	0	0	0	0	2	1	4
kinase	0	1	0	0	0	0	0	4
misc protein	0	0	0	0	0	0	0	3
transferase	0	0	0	0	0	0	0	2
ion channel	0	0	0	0	0	0	0	1
lyase	0	1	0	0	0	0	0	1

methyltransferase	0	1	0	0	0	0	0	1
Total	*27*	*18*	*14*	*11*	*21*	*43*	*24*	*1392*

Intended Target Family	CPF	CPF-O	DEET acid	Dieldrin	Heptachlor Epoxide	p,p'-DDE	p,p'-DDT	Total
cell cycle	31	30	1	53	123	74	120	487
nuclear receptor	26	24	2	34	32	102	58	318
DNA binding	21	12	1	10	24	64	27	172
cytokine	15	17	0	6	39	29	33	143
cell adhesion molecules	10	7	0	5	16	13	14	65
cell morphology	3	3	0	2	8	6	8	38
CYP	0	15	6	3	1	0	0	32
GPCR	3	7	0	2	2	4	4	25
protease	1	3	0	1	4	5	3	19
malformation	1	3	0	2	1	1	1	12
steroid hormone	6	0	0	0	0	0	0	12
transporter	1	3	2	1	1	1	1	11
histones	2	0	0	1	2	2	2	9
oxidoreductase	2	2	0	0	0	0	0	8
phosphatase	1	0	0	1	0	0	1	7
protease inhibitor	0	1	0	0	2	2	2	7
esterase	1	4	0	0	0	0	0	6
growth factor	0	0	0	0	2	1	1	5
hydrolase	0	0	0	0	0	0	1	4
kinase	0	0	0	0	1	1	1	4
misc protein	1	0	0	0	1	0	1	3
transferase	0	1	1	0	0	0	0	2
ion channel	1	0	0	0	0	0	0	1
lyase	0	0	0	0	0	0	0	1
methyltransferase	0	0	0	0	0	0	0	1

Total	*126*	*132*	*13*	*121*	*259*	*305*	*278*	*1392*

Table 19 NHANES Participant Demographic Frequencies based on Pesticide Measurement Bioactivity in Toxcast, 1999-2014

	Non-Bioactive		*Bioactive*			
	N=12,793		*N=8,549*			
Variable	*Mean*	*Standard Error*	*Mean*	*Standard Error*	*p-value*	
Body Mass Index	28.42	6.7	28.36	6.62	0.857	
Age in years	45.77	19.1	46.41	20	0.146	
Poverty-Income Ratio	2.58	1.65	2.42	1.6	5.82×10^{-6}	***
Survey Year	*N*	*%*	*N*	*%*	$<2.2 \times 10^{-16}$	***
1999-2000	251	1.96	1312	15.35		
2001-2002	201	1.57	1709	19.99		
2003-2004	1416	11.07	1832	21.43		
2005-2006	1285	10.04	401	4.69		
2007-2008	3143	24.57	570	6.67		
2009-2010	2873	22.46	1112	13.01		
2011-2012	2452	19.17	922	10.78		
2013-2014	1172	9.16	691	8.08		
Gender					2.4×10^{-3}	**
Men	6485	50.69	4142	48.45		
Women	6308	49.31	4407	51.55		
Racial Ethnicity					$<2.2 \times 10^{-16}$	***
Mexican American	1868	14.60	1927	22.54		
Other Hispanic	1080	8.44	533	6.23		
Non-Hispanic White	6297	49.22	3505	41.00		
Non-Hispanic Black	2499	19.53	2071	24.23		
Other Race	1049	8.20	513	6.00		

History of Farmwork					$<2.2 \times 10^{-16}$	***
Farmworker	475	3.71	662	7.74		
Non-Farmworker	12318	96.29	7887	92.26		
U.S. Citizenship					4.0×10^{-5}	***
Non-Citizen	1520	11.88	1262	14.76		
Citizen	11273	88.12	7287	85.24		
Country of Birth					4.94×10^{-12}	***
Born in 50 US states or DC	2660	84.36	4029	76.71		
Born in Mexico	231	7.33	754	14.36		
Born elsewhere	262	8.31	469	8.93		
Education Level					$<2.2 \times 10^{-16}$	***
Less than 9th grade	1166	9.13	1071	12.54		
9-11th grade	2299	17.99	1869	21.89		
Highschool	2975	23.28	1942	22.74		
Graduate/GED	3611	28.26	2198	25.74		
Some College or AA	2727	21.34	1460	17.10		

Significance: ‘*’p ≤0.05 ‘**’ p ≤0.01 ‘***’ p ≤0.001

P-values are derived from a Pearson’s chi-square test, using a Rao and Scott Adjustment where necessary. A Wilcoxon Rank test was used to test group means, with a Kruskall-Wallis Correction in low response categories.

Percentages are out of the total number of respondents for that specific question.

In this table, other race includes multi-racial.

In this study, 9-11 grade includes 12th grade completion without a high school diploma.

Table 20 NHANES Farmworker Demographic Frequencies, by Pesticide Measurement Bioactivity in Toxcast, 1999-2014

	Non-Bioactive		**Bioactive**			
	N=475		*N= 622*			
Variable	*Mean*	*Standard Error*	*Mean*	*Standard Error*	*p-value*	
Body Mass Index	28.2	6.05	28.4	6.11	0.514	
Age in years	48.65	19.2	48.61	18.4	0.979	
Poverty-Income Ratio	2.58	1.76	2.99	1.66	1.68×10^{-3}	**
Survey Year	*N*	*%*	*N*	*%*	$<2.2 \times 10^{-16}$	***
1999-2000	28	5.89	131	19.79		
2001-2002	17	3.58	202	30.51		
2003-2004	153	32.21	205	30.97		
2005-2006	17	3.58	15	2.27		
2007-2008	69	14.53	18	2.72		
2009-2010	105	22.11	49	7.40		
2011-2012	66	13.89	30	4.53		
2013-2014	20	4.21	12	1.81		
Gender					0.244	

Men	175	36.84	265	42.60		
Women	300	63.16	357	57.40		
Racial Ethnicity					0.134	
Mexican American	113	23.79	165	24.92		
Other Hispanic	16	3.37	20	3.02		
Non-Hispanic White	269	56.63	366	55.29		
Non-Hispanic Black	46	9.68	89	13.44		
Other Race	31	6.53	22	3.32		
U.S. Citizenship					0.044	*
Living with Citizenship	99	20.84	104	16.72		
Living without Citizenship	376	79.16	518	83.28		
Country of Birth					8.7×10^{-4}	***
Born in 50 US states or DC	187	86.98	419	75.77		
Born in Mexico	13	6.05	88	15.91		
Born elsewhere	15	6.98	46	8.32		
Education Level					5.0×10^{-4}	***
Less than 9th grade	121	25.47	112	16.97		
9-11th grade	69	14.53	78	11.82		
Highschool	94	19.79	116	17.58		

Graduate/GED	83	17.47	160	24.24
Some College or AA	108	22.74	194	29.39

Significance: '*'p ≤0.05 '**' p ≤0.01 '***' p ≤0.001

P-values are derived from a Pearson's chi-square test, using a Rao and Scott Adjustment where necessary. A Wilcoxon Rank test was used to test group means, with a Kruskall-Wallis Correction in low response categories. Row percentages are provided to show differences in sub-groups. In this table, other race includes multi-racial. In this study, 9-11 grade includes 12th grade completion without a high school diploma.

Table 21 Unadjusted Logistic Regression Models of Pesticide Bioactivity among Farmworkers and Non-Farmworkers

		95% Confidence Interval				
Chemical Name	Odds Ratio	lower limit	upper limit	X^2 statistic	p-value	
Beta-hexachlorocyclohexane	0.96	0.87	1.05	-0.92	0.454	
Dieldrin	0.99	0.90	1.09	-0.22	0.845	
Heptachlor Epoxide	0.98	0.89	1.07	-0.49	0.658	
p,p'-DDE	0.95	0.87	1.05	-1.02	0.403	
p,p'-DDT	0.98	0.89	1.07	-0.50	0.658	
2,4-Dichlorophenoxyacetic acid	1.38	1.23	1.55	5.33	1.69×10^{-6}	***
DEET Acid	1.80	1.29	2.50	3.49	1.60×10^{-3}	**
3-Phenoxybenzoic acid	1.49	1.32	1.68	6.37	7.47×10^{-8}	***
4-Nitrophenol	1.47	1.31	1.66	6.29	7.47×10^{-8}	***

3,5,6-Trichloropyridinol	1.50	1.33	1.69	6.50	7.47 x10^{-8}	***
Chlorpyrifos	1.50	1.33	1.69	6.50	7.47 x10^{-8}	***
Chlorpyrifos-oxon	1.50	1.33	1.69	6.50	7.47 x10^{-8}	***
2,5-Dichlorophenol	0.91	0.71	1.17	-0.70	0.538	
2,4-Dichlorophenol	0.91	0.71	1.17	-0.70	0.538	

Significance codes: '***' 0.001 '**' 0.01 '*' 0.05

These models are comparing farmworkers and non-farmworker pesticide measure bioactivity by the pesticide of interest and citizenship status. This model is adjusted for urinary creatinine for urine samples or lipids for serum measurements. Farmwork is defined as 1=history of farmwork and 0=no history of farmwork. Bioactivity is defined as 'positive' if the variable hitcall==1.

Table 22 Adjusted Logistic Regression Models of Pesticide Bioactivity among Farmworkers and Non-Farmworkers

		95% Confidence Interval		Fisher's Exact Test
Chemical Name	Odds Ratio	lower limit	upper limit	p-value
Beta-hexachlorocyclohexane	0.98	0.90	1.07	0.765
Dieldrin	1.00	0.92	1.08	0.953
Heptachlor Epoxide	0.99	0.91	1.06	0.817
p,p'-DDE	0.97	0.89	1.06	0.693
p,p'-DDT	0.99	0.91	1.07	0.817
2,4-Dichlorophenoxyacetic acid	1.04	0.92	1.17	0.693

DEET Acid	1.69	1.24	2.32	6.4×10^{-3}	**
3-Phenoxybenzoic acid	1.07	0.94	1.22	0.600	
4-Nitrophenol	1.07	0.94	1.21	0.600	
3,5,6-Trichloropyridinol	1.05	0.92	1.19	0.626	
Chlorpyrifos	1.05	0.92	1.19	0.626	
Chlorpyrifos-oxon	1.05	0.92	1.19	0.626	
2,5-Dichlorophenol	0.91	0.73	1.13	0.615	
2,4-Dichlorophenol	0.91	0.73	1.13	0.615	

Significance codes: '***' 0.001 '**' 0.01 '*' 0.05These models are comparing farmworkers and non-farmworker pesticide measure bioactivity by the pesticide of interest. This model is adjusted for citizenship status, body mass index, age, gender, racial ethnicity, survey year, creatinine or lipid, federal poverty-income ratio, and education level. Farmwork is defined as 1=history of farmwork and 0=no history of farmwork. Bioactivity is defined as 'positive' if the variable hitcall==1.

Table 23 Unadjusted Logistic Regression Models of Pesticide Bioactivity among Farmworkers, by U.S. Citizenship Status

		95% Confidence Interval			Fisher's Exact Test	
Chemical Name	Odds Ratio	lower limit	upper limit	t statistic	p-value	
Beta-hexachlorocyclohexane	1.42	1.33	1.52	10.16	7.09×10^{-12}	***
Dieldrin	1.51	1.39	1.64	9.61	1.28×10^{-9}	***

Heptachlor Epoxide	1.52	1.41	1.63	11.18	5.28 x10^{-13}	***
p,p'-DDE	1.42	1.33	1.52	10.07	7.09 x10^{-12}	***
p,p'-DDT	1.52	1.41	1.63	11.31	5.28 x10^{-13}	***
2,4-Dichlorophenoxyacetic acid	1.33	1.20	1.46	5.67	5.04 x10^{-7}	***
DEET Acid	1.00	0.87	1.15	0.00	0.996	
3-Phenoxybenzoic acid	1.32	1.19	1.45	5.47	1.61 x10^{-6}	***
4-Nitrophenol	1.30	1.18	1.44	5.23	3.10 x10^{-6}	***
3,5,6-Trichloropyridinol	1.35	1.21	1.50	5.53	1.69 x10^{-6}	***
Chlorpyrifos	1.35	1.21	1.50	5.53	1.69 x10^{-6}	***
Chlorpyrifos-oxon	1.35	1.21	1.50	5.53	1.69 x10^{-6}	***
2,5-Dichlorophenol	1.90	1.54	2.34	5.97	2.22 x10^{-7}	***
2,4-Dichlorophenol	1.90	1.54	2.34	5.97	2.22 x10^{-7}	***

Significance codes: '***' 0.001 '**' 0.01 '*' 0.05

These models are comparing farmworker pesticide measure bioactivity by the pesticide of interest and citizenship status. This model is adjusted for urinary creatinine for urine samples or lipids for serum measurements. Farmwork is defined as 1=history of farmwork and 0=no history of farmwork. Bioactivity is defined as 'positive' if the variable hitcall==1.

Table 24 Adjusted Logistic Regression Models of Pesticide Bioactivity among Farmworkers, by U.S. Citizenship Status

		95% Confidence Interval				
Chemical Name	Odds Ratio	lower limit	upper limit	X^2 statistic	p-value	
Beta-hexachlorocyclohexane	1.44	1.30	1.60	6.90	$9.75x10^{-7}$	***
Dieldrin	1.46	1.30	1.63	6.49	$4.44 x10^{-5}$	***
Heptachlor Epoxide	1.49	1.35	1.66	7.46	$4.16 x10^{-7}$	***
p,p'-DDE	1.44	1.30	1.59	6.95	$9.75 x10^{-7}$	***
p,p'-DDT	1.49	1.34	1.65	7.47	$4.16 x10^{-7}$	***
2,4-Dichlorophenoxyacetic acid	1.24	1.12	1.37	4.06	$4.14 x10^{-4}$	***
DEET Acid	1.03	0.89	1.19	0.33	0.817	
3-Phenoxybenzoic acid	1.25	1.14	1.38	4.49	$1.69 x10^{-4}$	***
4-Nitrophenol	1.24	1.12	1.37	4.26	$2.8 x10^{-4}$	***
3,5,6-Trichloropyridinol	1.28	1.15	1.42	4.55	$1.7 x10^{-4}$	***
Chlorpyrifos	1.28	1.15	1.42	4.55	$1.7 x10^{-4}$	***
Chlorpyrifos-oxon	1.28	1.15	1.42	4.55	$1.7 x10^{-4}$	***
2,5-Dichlorophenol	1.27	0.99	1.63	1.87	0.189	
2,4-Dichlorophenol	1.27	0.99	1.63	1.87	0.189	

Significance codes: '***' 0.001 '**' 0.01 '*' 0.05

These models are comparing farmworker pesticide measure bioactivity by the pesticide of interest and citizenship status. This model is adjusted for body mass index, age, gender, racial ethnicity, survey year, creatinine or lipid, federal poverty-income ratio, and education level. Farmwork is defined as 1=history of farmwork and 0=no history of farmwork. A person's measurement is considered bioactive if they have a measurement equal to or higher than the modl_acc value for a given chemical's active assay.

Chapter 5 Conclusion

5.1 Overview

Since pesticides were first used in the early 1910's use has increased exponentially over the decades to the point that today pesticides are considered essential to agriculture worldwide [12]. While use has increased, the research on how exposure to these chemicals affect human health has lagged [18]. Historically many chemicals, including those sprayed for human consumption, are used for years before adequate research proves the chemicals harm human health [18]. And once there is scientific proof the chemicals harm human health, it can often take years or longer to remove the chemical from the market for a specific country or region [18]. To stop this cycle of harmful chemicals being on the market, many researchers are moving to high throughput toxicity screening and integrated analysis of publicly available big data to more quickly and effectively understand how these pesticides effect the human body [19,20]. Through projects like the US multi-agency Toxicology in the 21st Century (Tox21) program, toxicologists are beginning to predict the mechanisms of action and health effects of more than 85,000 chemicals on the global market and making this data publicly available [19,20].

Social determinants of health—like occupation or citizenship—are social constructs that groups of people identify with and can alter the care people receive, their access to personal protective equipment (PPE), and has even been associated with increased pesticide exposure and health risks [74,77,139,143,173] . Farmworkers have significantly different health outcomes when compared to non-farmworkers, such as an association with higher prevalence and mortality rates of certain cancers, neurobehavioral disorders, and metabolic disorders [135–137,156–158]. Researchers have also identified marked increases in cancers, endocrine disruption, and neuronal disorders in migrant workers and other farmworkers living without citizenship in the U.S. [129,137,157,159,160]. When investigating environmental toxicants such as pesticides at a population-level, researchers must account for these social determinants of health since there exists a disparity in health markers due to oppressive systems on minoritized populations [79,105,139,173]. Moreover, among migrant workers, there tends to be limited access to healthcare, lower education, and lower pay which creates unique vulnerabilities for farmworkers without citizenship and who travel for contract farmwork[17,140,143].

Healthcare policy and services are limited to non-existent for immigrants and especially migrant workers residing in the US. Many policies that on the surface appear highly beneficial for the American people like the Affordable Care Act of 2010, actually exclude immigrants completely from accessing care [81]. In addition, agreements like the North American Free Trade Agreement between the US, Canada, and Mexico limit migrant worker rights [17]. Moreover, migrant worker health is often unprotected by the law and workplace discrimination leaves migrant workers very vulnerable [77,81,138,139]. Prior research on migrant workers in the US

Midwest found factors like economics, logistics, and health significantly affected the mental health of migrant workers [140].

Understanding farmworkers' perceptions of risk, and how they vary across different cultural and demographic groups such as education, will be essential for designing interventions to encourage PPE use and safe handling of pesticides. In chapter 2 of this study, none of the farmworker study participants residing in Northern Thailand wore chemical-proof aprons, chemical-proof gloves, or a respirator. The use of gloves, long sleeve clothing, and any sort of clothes covering (*e.g.,* rain poncho or plastic sheet) were usually used items with some damage and was not consistently used across workers. Our study reflects a myriad of other studies that have also found PPE use differs based on farmworker perceptions of risk, which can be heavily influenced by peers and societal norms [104,105,108–110].

This dissertation fills the gaps in the literature by quantifying population-level occupational pesticide exposure and the associated effects of social determinants on exposure and bioactivity. The goal of this dissertation is to investigate pesticide exposure concentrations among farmworkers versus non-farmworkers, and moreover, to understand how the concentrations present in these populations could affect human health. Chapter two presents the pesticide exposure and self-reported health outcomes of farmworkers and non-farmworkers residing in Northern Thailand. This chapter also includes detailed information on workplace air samples and behavioral observations. Chapter three samples publicly available data from the US National Health and Nutrition Examination Survey (NHANES) to better understand pesticide exposure stratified by social determinants of health, such as occupation and citizenship. Finally, in Chapter four, Toxicity Forecast Dashboard (Toxcast) data was analyzed to understand the biological activity of pesticides in the human body. Toxcast data was then compared to

NHANES data to better understand how bioactivity of pesticide concentrations present in blood and urine of NHANES respondents differed by occupation and citizenship status.

5.2 Chapter Two: Pesticide exposure and adverse health effects associated with farmwork in Northern Thailand

Agriculture is a major industry for Thailand and with the long growing season, agriculture is a year-round job [92,93]. Public health professionals can develop policy based on the proven health effects of these chemicals by quantifying pesticide exposures of farmworkers and non-farmworkers. Specifically, completing research in low- and middle-income countries (LMICs) like Thailand, can reflect how unique barriers to health are created for marginalized people especially those residing in LMICs. In the second chapter of this dissertation, a pilot project completed in Chiang Rai, Thailand, sampled farmworkers and non-farmworkers to understand pesticide exposure. Additionally, through administered survey responses, work observation logs, and air sampling we were able to quantify health symptomology and stressors, work conditions and practices, and pesticide exposure concentrations in the work area.

This study included 97 men between the ages of 22 and 76 years of age; 70 were conventional farmworkers and 27 did not report any prior farmwork [174]. None of the farmworkers included in this study wore standardized PPE for the concentrated chemicals they were working with. Methomyl (8.4-13,481.9 ng/m^3), ethyl chlorpyrifos (11.6-67,759 ng/m^3), and metalaxyl (13.9-41,191.3 ng/m^3) were detected via personal air sampling [174]. When it came to reporting confidence in the ability to handle personal problems, only 43% of farmworkers reported feeling confident, which reflects higher stress levels in comparison to 78% of comparison workers ($p = 0.028$) [174]. Farmworkers also had significantly lower monocyte counts (p=0.01), serum calcium (p=0.01), red blood count (p=0.01), white blood cell count (p=0.04), and butyrylcholinesterase activity ($p<0.0001$), relative to comparison workers [174]. After adjusting for Body Mass Index (BMI), age, and smoking, methomyl air concentrations were associated with a decrease in farmworker acetylcholinesterase activity (beta= -0.327, p =0.016) [174]. This population of farmworkers had significant alterations in stress measures and clinical biomarkers, including decreased blood cell counts and cholinesterase activity, relative to matched controls [174]. These changes are potentially linked to occupational pesticide exposures [174]. Improving PPE use presents an immediate likely route for preventive intervention in this population [174].

This is a new population of farmworkers who have not been studied before and is the first study to identify the use of face hats as PPE among farmworkers. Additionally, this is a community based participatory research study that was prompted from the farmworkers and included community members, health volunteers, hospital researchers, medical staff, as well as the farmworkers. It is ethically imperative to ensure that we are providing information and support to the farmworkers and making sure that we are supporting their needs from the research. Moreover, this study was novel by using mixed methods, including workplace observations, air samples, administered survey responses, and health biomarkers quantification.

An important next step includes creating focus groups among the farmworkers to understand the farmworkers' needs and perceptions of health risks due to pesticide exposure. Additionally, increasing the population size and sampling from different regions within Thailand can inform future public health policies put forward by the Royal Thai Government (RTG). It is important to understand that non-government workers, non-citizen, and migrant workers make up over 93% of agriculture workers in Thailand and are considered informal sector workers [45,94]. This means most if not all of the workers included in our study are not protected under the occupational health laws the Labor Protection Act [45,94]. By including more people, we hope to be able to stratify respondents by urban, rural, farming region, and occupation to create a more generalizable population and robust dataset on pesticide exposure in Thailand. Additionally, sampling farmworkers and residents in rural and urban areas could also be important next steps in creating region-related statistics to better understand differences in exposure and health of farmworkers and non-farmworkers residing in rural, suburban, or urban areas. Another important future direction is to assess pesticide exposure among migrant workers in Thailand from Laos and Myanmar.

5.3 Chapter Three: Assessing Pesticide Exposure among Farmworkers by US Citizenship in 1999-2014 NHANES Data

Historically pesticides have been produced and found to effectively cause death in rodents, insects, and unwanted plants [11]. Unfortunately, many of these pesticides also harm human populations once on the global market [7,10,11,126–129]. Public health professionals have focused on preventing exposure through research informed policy [18]. For example, the National Health and Nutrition Examination Study (NHANES) is a cross-sectional assessment of the health representative of adults and children residing within the US [175].

In this chapter, using NHANES data from 1999 to 2014, pesticide exposure biomarker concentrations were compared between people with and without a self-reported history of farmwork. This study categorized farmworkers by citizenship status to quantify the differential distributions of pesticide exposure between these groups. The detectable pesticides included in this chapter include 2,4-Dichlorophenol (24DCP), 2,4-Dichlorophenoxyacetic acid (24D acid), 2,5-Dichlorophenol (25DCP), 3,5,6-Trichloropyridinol (TCP), 4-Nitrophenol, β-hexachlorocyclohexane (β-HCH), diethyltoluamide acid (DEET acid), Dieldrin, Heptachlor Epoxide, 3-phenoxybenzoic acid (3-PBA), Trans-nonachlor, p,p'-DDE, and p,p'-DDT.

Overall, there were 1,137 people with any farmwork history and 20,205 with no farmwork history. Among the farmworkers, 203 reported not being citizens of the United States. When modeling concentration measurements and adjusting for social determinants of health, 2,4-Dichlorophenoxyacetic acid (24D acid) concentrations were 29.72% higher in farmworkers compared to non-farmworkers ($p=5.78x10^{-3}$). The herbicide 24DCP acid is a parent compound and phenol used in residential and commercial lawn and weed care and was also used in the Vietnam War as an ingredient of Agent Orange [142]. Broad leaf herbicides similar to 24DCP acid are most commonly used at home or a catch all killer for unwanted greenery [176]. When comparing farmworkers by citizenship, 2,5-Dichlorophenol (25DCP) (469.7%,p=0.029) and p,p'-DDT (150.23%, $p=3.0 \times 10^{-4}$) had the highest increases in chemical concentration among farmworkers living without US citizenship. This 1,4-dichlorobenzene metabolite, 25DCP, is the active ingredient in moth balls [40].

This study is the first study to compare pesticide biomarker concentrations present in blood and urine by both farmwork occupational status and US citizenship. This study also includes a large sample size and is very generalizable to the US general population. We identified disparities in pesticide exposure by farmwork history and US citizenship present in NHANES. Evidence-based legislation is needed to reduce these gaps in pesticide exposure and health. None of the health outcome data within NHANES was used within this chapter and would be a important set of data to integrate to further understand how these pesticides are affecting people who are exposed. Future research that compares NHANES should expand outcomes of interests to include health outcomes such as behavioral health conditions, cardiovascular disease, metabolic diseases, and cancer. It would be good to focus on these chronic health disorders since these are repeatedly correlated to pesticide exposure in the literature. Moreover, by focusing on publicly accessible demographic data and temporal pesticide exposure outcomes, researchers can quantify trends overtime in human biospecimens. This is imperative considering many of these chemicals can bioaccumulate and pesticide use trends have changed overtime. With more temporal research, researchers can also begin to understand how replacement chemicals affect population health over time compared to predecessor chemicals.

5.4 Chapter 4: Combining NHANES and Toxicity Forecast Dashboard Data to Compare Pesticide Exposure and Bioactivity, by Farmwork History and U.S. Citizenship

Many of the pesticides commonly used today were put on the market prior to any requirement for chemicals to prove they have limited effects on the environment or human body [18]. Both persistent and non-persistent pesticides have been associated with adverse health outcomes like diabetes, cancer, and birth defects [1,2,45,71,96,97]. The U.S. Environmental Protection Agency's Toxicity Forecaster (Toxcast) Dashboard, a new high throughput toxicological database, was spurred as part of Tox21 and recent updates in the US law [152]. Toxcast publicly reports high-throughput screening results on 8,200 chemicals and 1,192 chemical bioactivity assays that are categorized as either 'cell based' (*e.g.,* cellular viability or proliferation assays) or are 'cell free' (*e.g.,* enzyme or protein assays) [152]. By linking human population exposure data in NHANES to dose-response data from Toxcast, we can begin to estimate adverse biological effects that occur across a range of pesticide doses and exposures.

This study retained all NHANES study participants aged 18 years and older who also have occupation and pesticide exposure data present between 1999 and 2014. All 14 detectable pesticides and pesticide biomarkers in NHANES were compared to the positive assay concentrations of these same chemicals in Toxcast. We also duplicated the measurements of the chlorpyrifos (CPF) metabolite TCP to later compare to the Toxcast toxicity data for CPF and chlorpyrifos-oxon (CPO).We found NHANES participants are exposed to bioactive concentrations of pesticides, as identified by Toxcast high throughput screening assays. The most bioactive pesticides in Toxcast based on their overall percent of positive assays were heptachlor epoxide (39.85%), followed by p,p'-DDT (35.73%) and p,p'-DDE (26.78%). Overall, the top 3 assays which are commonly activated by the pesticides in NHANES were Attagene's gene expression assay for PXR target genes (PXRE_CIS_up) (N=11) and TOX21's mitochondrial membrane potential assays (MMP_ratio_down (N=11) and TOX21's _MMP_rhodamine (N=10), another mitochondrial membrane potential assay. Overall, our study found farmworkers without U.S. citizenship were more likely to have a bioactive measurement of heptachlor epoxide (OR=1.49, 95% CI: 1.35, 1.66) or p,p'-DDT (OR=1.49, 95%CI: 1.34, 1.65).

Assessing exposure is very complicated and is further complicated due to individual differences in metabolism and exposure, social determinants of health, most people being exposed to more than one chemical, and persistence of the chemical [131,164]. This is especially true with endocrine disrupting compounds since these represent a very diverse group of chemicals that are often measured based on their effect on the human cell [131]. A next step for this project would be a follow up study comparing intended targets from Toxcast to health symptomology reported in NHANES. This way, we can begin to quantify the relationship between bioactive pesticide concentrations in blood and urine, intended target family of toxicological assays, and actual population-level health outcomes.

Both Toxcast and NHANES are validated and reliable datasets within the field of public health and toxicology. This study is the first to directly compare NHANES and Toxcast data to understand human pesticide exposure concentrations by occupation and citizenship status. This study also considers social determinants of health and found statistically significant differences by social determinants of health in both pesticide exposure concentration and likelihood of being a bioactive biomarker measurement.

Additionally, future work should consider using predictive modeling to look at symptomology by social determinants of health and pesticide exposure levels. Many researchers are using predictive modeling techniques like artificial intelligence and machine learning to understand chemical toxicity [25,26,177]. These techniques have been quickly adopted in the pharmacology field, with the typical large pharmaceutical discovery project using between 20 and 50 different assays to collect human interaction data [25]. By utilizing machine learning techniques to compare health outcomes and intended target family by chemical, it is hoped that researchers can more effectively determine associated health effects of a chemical compound or part of a chemical compound.

5.5 Public Health Implications and Future Research Needs

The research within this dissertation answers gaps in environmental health research on pesticide exposure among the human population in Thailand, a middle-income country located in Southeast Asia, and the US, a high-income country in North America. In each chapter, farmworkers and non-farmworkers were exposed to one or more pesticides. In the final chapter, we learned both farmworkers and non-farmworkers in the US are exposed to bioactive concentrations of pesticides. Disparities in exposure exist when these cohorts were stratified by history of farmwork, US citizenship status, or racial ethnicity. Another exciting next step for the research presented within this dissertation, would be to compare Northern Thailand pesticide exposures in blood and urine to Toxcast outcomes. The literature still has limited human exposure data for many chemicals, especially when considering active and inactive ingredients.

Based on the data presented in this dissertation, farmworkers and non-farmworkers are exposed to pesticide concentrations that are harmful to human health. Farmworkers had differing health symptoms and stress levels in Northern Thailand. In our study, farmworkers in the US are disproportionately exposed to certain pesticides by occupation and for farmworkers by citizenship status. This dissertation contains new research of its kind linking occupational pesticide exposures with molecular mechanisms of action and health outcomes. Making these comparisons are important for shaping the future of environmental health research, law, and hopefully the way people interact with chemicals globally.

Researchers should also consider how bioactivity or chemical interactions are altered by both active and inactive ingredients. Chemical exposure and health hazards research often focuses solely on the active ingredients in the pesticide mixture. Pesticides on the market are predominantly inactive ingredients and therefore the way that these pesticides may affect the human body is also important [178]. Mixtures of pesticides, even at low doses, have been associated with endocrine disruption, carcinogenesis, cell proliferation, and other cellular perturbations [62,129,179,180]. As of 2004, the US EPA identified 3,000 inert pesticide ingredients that may have an effect on human health [178].

Prior pesticide research has found health harms associated with the pesticide mixtures on the market that are not replicable *in vitro* or *in vivo* with just the active ingredient [178]. For example, researchers investigated the Roundup branded pesticide mixture versus its active ingredient alone, glyphosate, effects on the enzyme aromatase [181]. Aromatase catalyzes the body's conversion of androgens to estrogen [181]. These researchers found aromatase to be consistently inhibited in human placental cells exposed to Roundup [181]. More specifically, Roundup inhibited aromatase by roughly 30% more at a higher concentration (0.02% Roundup versus 0.04% Roundup) [181]. However, these results were not found consistently in glyphosate alone, with many of the experiments showing no inhibition in aromatase [181]. For this reason, future public health research on pesticide toxicity should include relevant commercial mixtures to best understand the mechanisms for human health outcomes. This is an important next step because farmworkers are being exposed to pesticide mixtures and often are the ones mixing the concentrated chemicals and we need to understand how these unique exposures may be associated with health harms.

In conclusion, researchers, the public, and law makers must understand that pesticides are here to stay. Even if all pesticides were taken off the market today, there would still be surplus stocks to be used for some years to come based on previously banned chemicals in the US and Thailand. Pesticides, among other chemicals, are still placed on the global market with very limited publicly-available human health research accompanying these chemicals. This lag in publicly available research has left public health research far behind. More research on how the chemicals on and in our clothes, food, animals, and bodies is imperative to the health of all people, especially the most disenfranchised.

Appendix A List of Pesticides Included

a. Persistent Organic Pollutants (POPs)

i. Organochlorines (or chlorinated hydrocarbons)

1. DichlorDiphenylTrichloroethane (DDT)
    a. *Metabolites*: p,p'-DDT | p,p'-DDE
2. Lindane
    a. *Metabolites*: β-hexachlorocyclohexane
3. Chlordane
    a. *Metabolites*: Oxychlordane | Trans-nonachlor

ii. Chlorinated cyclodienes

1. Dieldrin

iii. Heptachlor

a. *Metabolite*: Heptachlor Epoxide

b. Non-Persistent Pesticides (NPPs)

i. Organophosphates (AChE Inhibitors)

1. Chlorpyrifos (CPF)
    a. *Metabolites*: Chlorpyrifos-oxon (CPF-O) | 3,5,6-Trichloropyridinol (TCP)

ii. Chlorinated Phenols

1. 1,4-dichlorobenzene (moth balls)
    a. *Metabolite*: 2,5-Dichlorophenol (25DCP)
2. 2,4-Dichlorophenoxyacetic Acid (24D acid)
    a. *Metabolite*: 2,4-Dichlorophenol (24DCP)

iii. Carbamates

1. Methomyl

iv. Acyl Alanine Fungicide

1. Metalaxyl

v. Pyrethroids

1. 3-Phenoxybenzoic acid (3PBA)

vi. Toluene

1. N,N-diethyl-meta-toluamide (DEET)

b) *Metabolite:* N,N-diethyl-meta-toluamide acid (DEET acid)

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